the science of beauty

Vol 9 No 4

January 2020

53rd ASCC Annual Conference

19th - 21st May 2020

Crown Conference Centre Melbourne

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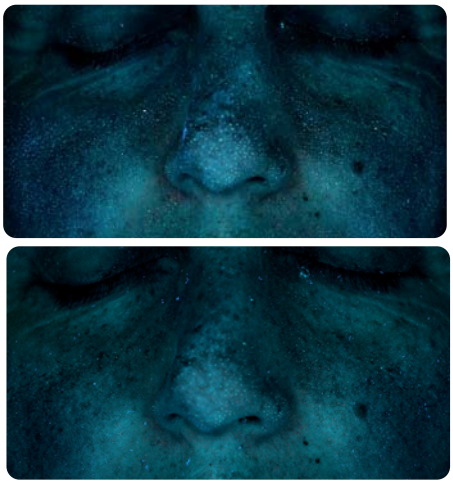

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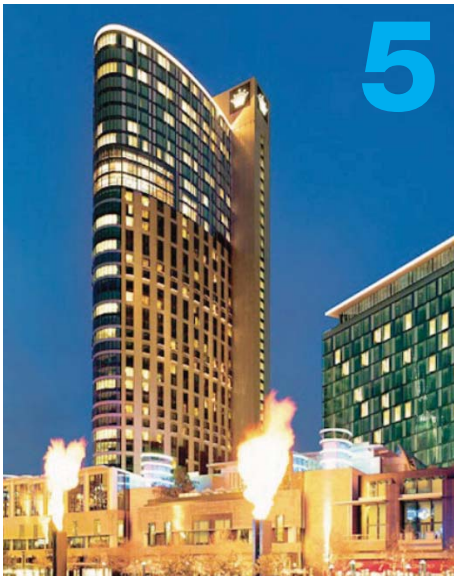
ASCC



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FOR A CLEAN AND SUSTAINABLE FUTURE

CROWN CONVENTION CENTRE, MELBOURNE, VICTORIA, AUSTRALIA

19th-21st May, 2020

THE COUNTDOWN IS ON – ONLY 3 MONTHS TO GO FOR ASCC 2020!

Welcome back to everyone for 2020 and I hope you had an enjoyable Festive period. Unfortunately the summer months in Australia have and will continue to be a difficult time for many due to devasting effects of the recent bushfires throughout many parts of this great country. Our thoughts and prayers certainly go out to those affected as they struggle to regain a sense of normality after an incredibly difficult time. A special thank you must go out to all the thousands of volunteers helping in any way they can both on the front line and supporting the many intiatives in place. It is times like these in the face of extremely difficult situations that the genorosity and belief of everyone really shines through.

As we approach our upcoming conference in May these thoughts will continue to be with us, and with our Conference theme centred around Sustainability maybe this is the time for our industry to really focus on the impact we are having on the wider environmental situation. By bringing Sustainability and a Cleaner future front of mind it might be the chance we have to make some long term and beneficial changes that can have wide reaching implications.

We have lots of news to share with you for this update:

- Our Technical Program has been finalised. Along with our Keynote Speaker, John Warner, we have 2 Plenary Speakers, 48 platform presentations, 6 workshop and 1 live Panel discussion continuing the success over the last few years.
- Our Plenary Speakers will be Jess Van Zeil, best selling author and resillience expert and Travis Badenhorst, Chief Scientist and Global R&D Leader for Snowberry NZ. Both will offer their expertise and how Sustainability influences their decisions everyday.
- There are still some great general sponsorship opportunities available for companies looking to step up their visibility and become involved in our conference. Make sure you check out the Sponsorship Prospectus on the ASCC website to get involved!
- The delegate registration package is now on sale. Earlybird registrations will close on 29th February so make sure you register early to avoid missing out on access to the social events. You can find the booking form for the conference at https://events.ozaccom.com.au/ascc-2020/registration.

I would like to thank all our Premium Sponsors, General Sponsors, Exhibition Booth holders and Confirmed presenters. Without you this conference will not happen and we thank you all for your support. To the Conference Organising team this conference is a testament to hard the hard work and determination you have all put in over the last 12 months.

Matthew Martens – ASCC 2020 Conference Chairperson

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To keep updated with all the latest conference information make sure you visit www.ascc.com.au

A.S.C.C. PULCHRITUDO PER SCIENTIAM

52nd Australian Society of Cosmetic Chemists Conference

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meet the team...

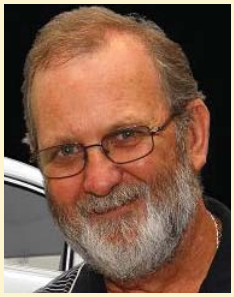
WENDY FREE has degrees in Science (B.Sc) and Technology Management (M.Tech Mngt) and is a member of a number of industry associations including Australian Society of Microbiologists, Royal Australian Chemical Institute, Association of Therapeutic Goods Consultants and is a Fellow of the Australian Organisation for Quality. With more than 25 years industry experience, Wendy's current roles include APVMA GMP auditioning, contributing to the Cochrane Collaboration and on a day to day basis, Scientific Director Quality Matters Safety Matters Pty Ltd (QMSM) that has over the last decade Wendy has provided expertise to over 400 Australian and International businesses. She specialises in regulatory compliance, commercialisation, troubleshooting and GMP systems, and considers cosmetics amongst the most challenging and enjoyable part of her work.

JULIAN JONES, the founder and Managing Director of ikonsulting Pty/Ltd, is Passionate about the Personal Care Industry in Australia and Globally.
Julian has been an active member of the ASCC for over thirty years. During this time he has served as President and Chairman of the Victorian Chapter of the ASCC. He is widely known and well respected both nationally and internationally for his knowledge and skills in developing and marketing the best Personal Care Products.

JOHN STATON has a background of over 40 years experience in the pharmaceutical and healthcare industries. John is a life member of the ASCC and serves in a number of industry representative roles with ASMI, ACCORD, TGA and Standards. He is the Australian representative to the ISO Committee on Sunscreen Testing-TC 217. (The committee for development of sunscreen standards). John is also in demand as a speaker on the International Conference Circuit.

TONI OVENELL is a formulation chemist and consultant for Queensland Cosmetic Formulators. She has worked in the cosmetic industry for many years in a range of roles covering areas of technical sales, quality, supply chain, manufacturing and product development. Most recently Toni has worked for a small contract manufacturer as technical manager, prior to setting up her own business. Toni is passionate about sharing her knowledge, maintaining a viable cosmetic industry in Australia and helping people bring their product ideas to market. She also likes champagne and hockey.

PAM JONES has worked in the Personal, Homecare and Pharmaceutical markets for more than 30 years. She has been working out of Asia since 1996 and is well versed and connected with the Asia Market.
Her experience covers technical, sales, marketing, management and training roles. She has qualifications in Chemistry, Marketing and Management. Her company PCA Consulting is well known for its training programmes. Pam has worked with and consulted to companies such as ICI, Croda, Ashland, Huntsman, Reed Exhibitions (in Cosmetics) and Connell to name a few. She is currently serving on the ASCC Technical Committee and volunteers as Technical Editor for this magazine.



RIC WILLIAMS was educated in Sydney obtaining his Bachelor of Science in Pure and Applied Chemistry from the University of New South Wales (1980) and a Diploma of Environmental Studies from Macquarie University in 1983.
Ric has had 40 years experience in the industry working for many companies and operating his own consultancy business for many years.
He has presented many lectures and workshops at national conferences for the Australian Society of Cosmetic Chemists (ASCC), the Association of Professional Aestheticians of Australia (APAA), Cosmetic and Pharmaceutical Special Interest Group (CAPSIG) and also beauty colleges nation wide.

MARG SMITH is the owner of Syndet Works – an Australian company established in 1984 to formulate and produce soap free skincare bars. Syndet has developed an enviable reputation for custom formulated and manufactured skincare that now extend well beyond the origins of the business.

JEN SEMPLE is Innovation & Education Manager at Accord Australasia, the peak national body for formulated chemical products. She is passionate about communicating the benefits of our industry's products to wider society and has authored a number of public education websites such as furphies.org.au, sunsible.org.au and hygieneforhealth.org.au. Jen also manages Accord's sustainability initiatives and seeks opportunities to build relationships between industry and academia. She has a PhD in Chemistry and Graduate Diploma in Education, and is a member of the Royal Australian Chemical Institute.

EMANUELA ELIA is the Director of Ozderm, which specialises in *in vivo* testing and clinical trials for cosmetic and personal care products. Emanuela Elia has a law degree from Rome and a Master of International Business from the University of Sydney. She had collaborated with Australia's longest serving Contract Research Organisation Datapharm for a few years before setting up a cosmetic and personal care products testing facility in 2009. Emanuela is enthusiastic about improving the quality of cosmetic and personal care products' research in Australia through science.

JAMES GILLARD is the Principal of Insurance Made Easy whose services include – business insurance, travel insurance and financial services. Insurance Made Easy has a client list of over 2000 businesses from all industries. The relevant major insurance schemes are – Hair and Beauty, Pharmaceutical Companies and Natural Therapists.

STEVE WELSH is a cosmetic packaging specialist with over 20 years experience across all mediums of packaging. As the director of Weltrade Packaging, Steve leads a team of designers, technicians, printers and supply chain professionals. To ensure the best exposure of your beauty, skincare or cosmetics brand. Steve's philosophy is to design your packaging correctly, right from the start, so you can elevate your brand and move more product. Steve works closely with leaders in the cosmetic industry to ensure that your packaging consistently stands out on the shelves within this highly competitive market.

TINA ASPRES has worked as a Pharmacist for almost 20 years in retail, industry and academia as well as being a Cosmetic Chemist. Currently she works in industry and has vast experience in both the pharmaceutical and healthcare arenas. In addition to this she is a casual academic at UTS, School of Health, (Faculty of Pharmacy in Pharmaceutics). Tina has a great interest in clinical research in dermatology and the treatment of skin disease and conditions and is Clinical Trial Coordinator at South West Sydney Dermatology. She is a keen researcher in transdermal drug delivery systems. Tina is a Member of the Pharmaceutical Society of Australia and a Member of the Australian Society of Cosmetic Chemists. She regularly consults pharmaceutical companies in the area of acne, eczema and skincare especially in the area of cosmeceuticals and has devised and written numerous support, training and education material for companies aimed at both professionals and consumers. Tina consults for the Eczema Association Australasia and is on their Integrity Assessment Panel and has worked with Choice Magazine on numerous reports. Tina has presented at the Annual Scientific Meeting of the Australasian College of Dermatologists and has published within the pharmacy and medical literature in the area of sun protection, Vitamin D, skin cancer prevention and eczema as well as co-authoring the book 'All About Kids' Skin – The Essential Guide' published by ABC Books

GINT SILINS is a registered patent and trade marks attorney, and a principal of Spruson & Ferguson Patent & Trade Mark Attorneys (incorporating Cullens). He holds a Bachelor of Science degree in chemistry with honours in biochemistry, and a Doctor of Philosophy degree in biochemistry. Gint specialises in protecting branding and innovations largely in the health care, personal care, animal health, food and beverage, biotechnology, industrial chemical, clean energy and agricultural sectors. His practice includes: conducting brand and innovation availability and registrability searches; IP audits; registering patents, trade marks and designs worldwide; enforcing intellectual property rights; resolving IP disputes; and, providing infringement and validity advice.

embracing change…

like it or not!

by Julian Jones

As we commence a new year and a new decade, one thing is certain in a very uncertain future……change! The "rules" of marketing that seemed to apply for a very long time are being tossed out as consumers are increasingly not playing by them. The days of developing a product range, getting it onto shelves and then throwing a massive advertising budget at it to get things selling just doesn't work anymore.

Sure, if you're Coca Cola or Apple, constantly putting your brand in front of consumers continues to pay dividends but if you are a new brand trying to generate some customer interest, dollars aren't the magic wand they once were. Couple that with an increasingly better educated consumer base who are also less and less susceptible to traditional marketing campaigns and you, as a brand owner face a huge challenge to grow a successful business.

Some people say this change in marketing has been a long time coming and "lazy" brands only have themselves to blame. Overhyped products with unsubstantiated benefit claims have resulted in a jaded, cynical customer population who are more likely to question your marketing than accept it.

So, how do we rise to this challenge and leverage it into opportunity?

Well, one controversial way is to try being honest with your clients! Don't over promise and under deliver. On the contrary, delight them with better results than they expect. It's not that hard to do if you and your team genuinely hold the customers' best interests at heart. Understanding their expectations and empathising with their lives can lead to incredible brand loyalty and a legion of unpaid sales reps who are eager to spread the word about your amazing products and brand.

Putting your products in front of potential customers requires a very good understanding of your customer demographic. Their age range, home and work environment, working hours and days, free versus committed time amongst many other pieces of information all help you to provide the ideal solution to their beauty care needs and wants. Collecting all this information can be achieved by asking your existing customers if they are prepared to tell you about themselves and you'll be surprised how open they are to this if you treat them with respect and a genuine interest in them. If you don't have existing customers, have a look at other brands and products that are targeting the same type of customers as the ones you are interested in and learn what is working for them.

As you have probably worked out by now, growing your brand using these techniques does not happen if all of your marketing is broadly spread and lacking focus. Today's consumers, particularly millennials, all believe they are the centre of their universe and deserve to be treated that way! Whether this is a realistic life view is open to debate but that's what brand owners are facing for now so mass personalisation is the new rule of marketing. Mass production is the only way to get economies of scale and competitive customer pricing, but the consumer experience must feel bespoke and intimate.

Work out how to address this paradox and you are well on the way to being a successful 2020's brand!

Till next time – in 2020! Cheers,

Julian

- Sunscreen Testing
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- Chemistry
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BREAKING NEWS:

Cosmetic Packaging that BIODEGRADES in landfill WILL be a game changer

by Steve Welsh

At Weltrade Packaging we don't like to sit on our hands and do just the bear minimum, we like to be ahead of the curve in terms of sustainable packaging for the cosmetic and wellness industries.

Since our inception over 15 years ago, we have coached our clients on the importance of recyclability, providing alternatives, education and encouragement to look past products that won't be accepted in curb side recycling programs and are not BPA free, and offered 'greener' alternatives.

We have been working with bioplastics derived from plant based ethanol for over two years. Our sugar cane polyethylene tubes have come a long way with testing and now allow for aggressive ingredient reaction with the incorporation of an EVOH oxygen barrier to stop gasses from forming and discolouring. Meaning they work great for sunscreens and other applications.

When brands like Coke and Nestle said they were moving to PCR (post-consumer resin) for their packaging, we had been doing it with our clients for over 18 months. The number speak for themselves, consumer driven, increased unit sales even at slightly higher unit prices. Our resins are reclaimed packaging that could have possibly ended up in landfill or ocean waste. The ratio of PCR to virgin plastic material has increased consistently to make sure the functionality and colour is not lost as a result of a higher recycled content.

Packaging reduction is also a real push for brands, we help by looking at ways of reducing the amount of their packaging through better design and lighter parts. We introduced pouches and made sure

that the packaging is as sustainable as possible, including recycled paper as a component of our kraft paper pouches.

In the last 25 years in the packaging industry, what we have not been able to counter is the amount of goods that are actually recycled in Australia. Latest figures put the percentage around 13% of total plastic goods that are recycled. Despite our best efforts, 87% goes into landfill or ocean waste.

Mid last year we came across a technology that allows an additive to be added to our normal packaging. This additive does not discolour the base material, it does not taint or alter the chemical resistance stability. It does not affect the functionality of flip top or disc top or tamper evident caps/closures and it can be added to most types of plastics such as PET, HDPE and Polypropylene.

What this additive does do, is that it revolutionises what happens to the packaging when it goes into waste stream or the 87% that was discarded and was sitting in landfill for over 100 years for our children's children to deal with. After extensive testing and independent laboratory trials we can now supply packaging that will biodegrade in land fill in a much shorter timeframe. Not only will it break down all commercial packaging resins, it also leaves no micro plastics that will leach out into the environment.

Depending on the style of landfill management, the items can break down in as little as 6 years. With an investment of around 12% to packaging component, any responsible brand would be negligent not to take a stand and make sure their packaging is not left for future generations.

We will be publishing the independent findings and more technical information on our website over the coming weeks, so be sure to follow our news or social media channels. It really is time to have a conversation with our team and take action to deal with packaging responsibly, through recycling, recycled, reducing and NOW bio-degrading. For more information please visit weltradepackaging.com.au or give us a call on 07 55970102.

STEVE WELSH is a cosmetic packaging specialist with over 20 years experience across all mediums of packaging. As the director of Weltrade Packaging, Steve leads a team of designers, technicians, printers and supply chain professionals. To ensure the best exposure of your beauty, skincare or cosmetics brand. Steve's philosophy is to design your packaging correctly, right from the start, so you can elevate your brand and move more product. Steve works closely with leaders in the cosmetic industry to ensure that your packaging consistently stands out on the shelves within this highly competitive market.

why is treatment risk insurance so important?

by James Gillard

Most consumer claims from cosmetic products recorded by the ACCC relate to people suffering from some sort of mild skin irritations .There are also cases of serious consumer reactions such as hair loss, allergies, breathing problems, etc., which can be from make up, perfume and personal care products.

Recent improved labelling of cosmetic products has no doubt helped the Industry to reduce the incidence of these events occurring as consumers become more educated to what they are buying and using.

Compliance with the mandatory standard for cosmetic ingredient labelling is crucial as detailed on the Product Safety Australia website (www.productsafety.gov.au). This means the people who make and supply cosmetic products whether they are a manufacturer, wholesaler, supplier, or retailer must be fully aware of their responsibilities.

The information available on the Product Safety Australia website covers how to maintain consumer confidence and prevent consumer claims from Cosmetic based products. It helps consumers identify the presence of ingredients that they may be allergic or sensitive to, or otherwise concerned about, and it provides a comparison of different products at the point of sale. An inaccurate list of ingredients, or a product without an ingredient list could see consumers with allergies exposed to harm. The cosmetics labelling standard was developed so everyone buying and using your products knows what is in them.

Past industry compliance activities have been undertaken to understand why the injury reports from the exposure to or application of cosmetic products are so high and to survey the market for

compliance with the cosmetics labelling standard.

The main observations have been;

- many of the mandatory reports involved injuries from face washes / creams, body wash products and creams, and deodorants
- some products had ingredient labelling that was inconsistent with their formulation lists, or displayed their ingredients list in a way that could not be considered to be prominent or legible (as required under the cosmetics labelling standard)
- surprisingly low mandatory injury reporting rates were recorded for pharmacies, retail stores, department stores, and
- Australian based online sellers recorded no mandatory injury reports during that period, despite occupying an increasing proportion of sales in cosmetics in recent years.

Where Insurance Maybe Important

Treatment Risk Insurance

Although the above disciplines are in place there, is still a chance for a consumer to take legal action against your Business due to the liability associated with failed treatments or their reactions to products you supply. It is therefore important that your Public Liability insurance extends to cover these circumstances. Failed treatments may include cosmetic procedures such as hair coloring, chemical peels, hair removal, eye lash tattooing, skin infection following a procedure, and any client complication which arises following a treatment.

You might find that your standard Public Liability insurance excludes liability arising from professional risks such as advice, design, specification or treatment. Make sure your insurance policy includes treatment risk.

Other Insurances which may be applicable

- **Business Insurance for Retailers**

This Insurance covers your Buildings, Stock/ Contents, Business interuption, Theft, Glass , Money, Cyber Insurance, and General Property Insurance for All Risks (e.g. Mobile Equipment such as laptops, mobile phones which are stolen from a car, or accidentally damaged within Australia)

- **Insurance for Industry sponsors, manufacturers, distributors, retailers, and technicians**

As an industry specialist consultant

If you offer services including design, advice or formula including advice and assistance, you should have the right Professional Indemnity and Public Liability Insurance. Professional Indemnity Insurance covers the legal liability for claims arising out of an actual or alleged breach of your professional duty wheras Public Liability Insurance covers you against 3rd party personal injury, loss or damage to property as a result of an occurrence happening in connection with your business.

Other Insurances might be needed such as.

- Management Liability insurance &
- Cyber Insurance

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James Gillard

Managing Director

'A global marketplace – local & international opportunities and responses'

Save the date! Accord Cosmetic & Personal Care Conference 2020

26 November 2020, Sydney Harbour Marriott

by Jennifer Semple

Accord's biennial Cosmetic and Personal Care Conference is an industry highlight. Last held in 2018, here's what some attendees said about the event:

'A not-to-be-missed gathering'

'A fantastic conference with great insights into the cosmetic and personal care industry'

'Best conference ever!'

So…

Lock in the date: 26 November 2020 to focus on the challenges, opportunities and responsibilities of our industry in the context of a global business environment.

The programme will comprise international speakers and local experts, covering topics relating to:

- Industry's role and responsibility, including social licence to operate, strategies to address pressing environmental challenges, and maintaining impeccable standards of safety and compliance
- Latest global trends, including megatrends relating to our industry, new social media (what on earth is TikTok?), and the recent phenomenon of 'A-beauty'
- Local updates relating to key

Cosmetic Conference 2018 – Mike Rae, Sharan Kwek, Tracy Raso, Ellie Kim, Samantha King, Andrea Ferrari, Terry Little – all sponsors.

Cosmetic Conference 2018 – room, wide shot.

developments in the Australian business environment and from Australian business leaders

Accord's Conference will attract key personnel from the cosmetic and personal care and related industries – some for networking, some for knowledge, some for inspiration – and some for all the above.

Expect to be engaged mentally and stimulated socially – all while enjoying the excellent comfort and service provided at the Sydney Harbour Marriott.

A glimpse at the 2018 Conference – Future-proofing our industry

To whet your appetite for the 2020 event, let's have a brief look back to Accord's 2018 Conference, which was all about the Cosmetic and Personal Care sector taking control as we examined the threats and looked at opportunities created by a dynamic, ever-changing global environment.

International speakers included Cosmetic Europe's John Chave and PCPC's Lisa Powers, who spoke about their respective organisation's approaches to current and emerging industry issues. Local presenters gave fascinating and informative presentations on topics including:

- megatrends relevant to the cosmetics and personal care industry (by Mintel)
- what makes Millennials tick, disabusing some audience members of their misconceptions regarding this influential generation (by Junkee Media)!
- what Australian consumers value (by Roy Morgan Research)
- changes to consumer law and how they impact our industry (by Thomson Greer)
- using procurement spend to deliver social impact (by Social Traders)

There were also two panel discussions comprising local industry experts that explored *Strategies to future proof your brands, and Meeting consumer needs and wants.*

More details about this year's event will be provided in the coming weeks. Alternatively, please contact Stephanie Hollands on shollands@accord.asn.au or 02 9281 2322.

The conference is open to members and non-members of Accord. We look forward to welcoming you there!

Accord Australasia is the peak body representing companies operating in the cosmetic, fragrance, personal care and toiletries sector – from multinationals to small Australian-owned businesses, importers to local manufacturers. www.accord.asn.au

how much does a cosmetic study cost?

by Emanuela Elia

When cosmetic companies evaluate the potential to conduct an in vivo scientific study or consumer study on a skin care product, one of the major deciding factors is naturally its cost. It is no surprise then, that research service providers like us spend a lot of time discussing this with clients! When clients are fairly new to conducting research, it is not uncommon for them to ask for an estimate even before study parameters have been determined.

For example, understandably, cosmetic companies may ask for a 'price list' where they expect to pick and choose various aspects about their study with associated prices for each item. However, it is difficult for research companies to produce such a list as they are not selling standard services. Rather, each study is unique and the work involved will be tailored services specific to the needs of the client. While the ultimate goal of contracting a clinical trial site is to get the research done, this is achieved through the man-hours that take to complete the project, the use of the equipment to conduct the assessments and the maintenance of it, as well as engaging their technical expertise and experience. Furthermore, it is important to keep in mind that all these aspects interact together, meaning that cost cannot simply be determined by adding up these aspects in isolation.

As a general rule of thumb, there are certain aspects of a study that will cost more than others. It is important for companies to be aware of this when designing their study, and we hope that the information provided here will give a basic understanding of what parameters influence the cost of a study and why that may be. By becoming more familiar with these aspects, companies will have a better idea, roughly speaking, of the costing associated with possible cosmetic testing in vivo.

Here is a list of the main elements and variables that are likely to make up the cost of a study:

Types of assessments

Based on the aim of the study, there are certain assessments that will be more suitable than others. Generally, cost decreases as one goes down the list:

- Digital photos (i.e. before & after comparisons) require a professional photography set up in order to take images with consistent lighting, exposure, distance, and angle to subject. Photos are mainly used for comparison purposes in order to visually identify changes in certain skin parameters (e.g. skin colour and texture). Additionally, image analysis can be performed to provide additional data.
- Objective measurements often involve instruments or other validated techniques used to objectively assess. For example, to quantitatively determine any changes in skin parameters (e.g. improvement in skin elasticity by x%).
- Expert assessment involves assessing changes in skin parameters through training and validated scales (i.e.

expert's opinion). Please note that depending on the expertise and qualifications of the expert, this assessment could be more expensive than digital photos or objective measurements. While it is not as an objective measure when compared to photos or instruments, it is recommended that their use in cosmetic studies should be limited to when objective assessments are not possible due to the nature of the outcomes measured.

- Subject questionnaires are based on the perception and opinion of the person taking part in the study and is therefore not an objective assessment. However, it allows to collect data on the likely opinion of future consumers with regards to various aspects such as perceived likeability, efficacy, and safety of the product (e.g. x% of people liked product consistency or x% thought their skin was softer)

Other study parameters

This section describes other aspects of the study that need to be considered. In general, cost is consummate with complex and time spent:

- *The type of analysis performed on the data:* Certain assessments, like objective measurements require more complex statistical analysis including QC of tables and figures.
- *The number of participants in a study:* this is a 'sample size' and is based on the aim of the study, the type of assessment, and the magnitude of the expected product effect. For example, if the aim of the study is to prove an improvement in skin hydration after x number of days/weeks, a certain number of participants should be included in the study. If the aim of the study is instead to prove a change in elasticity, the number of participants included in the study would likely be different. More participants will require more time from the study staff to properly screen and assess.
- *Number of visits:* dependent on the amount of expected change over time and the length of the effect being measured, the number of times a participant will vary. Each visit involves the study participant presenting to the scheduled appointment so the required skin assessments can be conducted. Studies will usually involve at least three visits or more if objective measurements are involved. However, where the primary aim of the study is to collect some consumer feedback, only two visits might be sufficient. Studies can have as many visits as needed to be able to collect the evidence required to support the desired claims.
- *Duration of each visit:* depending on the type of assessment and the number of assessments, duration of the visits can vary. Longer visit times will mean that less participants can visit on any one day.
- *Preparation of study documents and setup costs:* these are part of the study set up. Large studies (i.e. more assessments, more visits, more participants) will involve more preparation. If regulatory bodies are involved, this will also significantly increase the time required.
- *Difficulty of recruitment:* When inclusion/exclusion criteria are very selective (i.e. women with eye puffiness aged 20 to 30) or the study requirements are quite demanding (e.g. several visits, long visits, study procedure at home or in clinic is complex), less people are likely to be eligible or willing to take part. This in turn means more time will be spent on recruitment, with additional advertising being involved.
- *Volunteer travel and time reimbursement:* although subjects volunteer to be involved in a study, generally a travel reimbursement and/or small compensation for their time is provided. Compensation given is often relative to the number of visits and how difficult it is to follow study requirements.

Conclusions

We have so far identified the main components that determine the cost of a study. It is important to note that when multiple of the same element are included in the study, reductions can apply based on economies of scale. That, in conjunction with compounding interactions between aspects of the study means price per element is almost impossible to determine. Rather, a study must be looked at as a whole.

As a cosmetic company, it is important to understand that determining study cost starts with the study synopsis based on the desired outcome. This will detail the aims of the study, intended number of participants, number of visits, type of assessments, and so on. Unfortunately, the conversations between cosmetic companies and researchers start the other way around (i.e. with this cost, what study design can we have). These can be quite counterproductive for most clients! As with any other project type service, keeping the conversation on the aim of the study is the only way to figure out the costs involved.

EMANUELA ELIA is the Director of Ozderm, which specialises in *in vivo* testing and clinical trials for cosmetic and personal care products. Emanuela Elia has a law degree from Rome and a Master of International Business from the University of Sydney. She had collaborated with Australia's longest serving Contract Research Organisation Datapharm for a few years before setting up a cosmetic and personal care products testing facility in 2009. Emanuela is enthusiastic about improving the quality of cosmetic and personal care products' research in Australia through science.

faking it with dihydroxyacetone

by Tina Aspres

Prior to the 1900's, fair skin was associated with belonging to the upper social class and the perception of status and wealth. People would go to great lengths to lighten their skin using various bleaching and skin lightening agents. Come the 1930's the tide changed, and all of a sudden advertisements promoting holidays in the French Riviera and Coco Chanel sporting a tanned look appeared, and tanning soon became associated with wealth, status, improved self-esteem and body image, and thus the desire to have a tan became more desirable. With an increase in tanning as people exposed their skin to ultraviolet (UV) light with minimal to no sun-protection, the incidence of skin cancer increased rapidly over time.

It is a well-known fact that UV radiation is a carcinogen. The warning of the dangers of UV radiation exposure from the sun and the associated risk of developing skin cancer are constantly echoed by the Cancer Council Australia and the Australasian College of Dermatologists. Unprotected exposure to UV radiation can cause an increase in the incidence of basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and melanoma as well as contributing to the effect of accelerated skin aging and wrinkling (photoageing).

Despite the greater awareness of the harmful effects of the sun on the skin, the desire to look tanned, especially during the summer months, continues to be coveted by individuals. The pursuit for a safe, 'healthy', 'golden glow' has led to consumers to look at alternative, safer methods without the risk to achieve that coveted and desirable sun-kissed glow. With this pursuit, self-tanning products have become very successful cosmetic products, with a steady growth rate seen in the category each year.

Self-tanning products have been developed as a safer alternative to provide skin colouration for the face and body – similar visibly to the tan achieved from exposure to UV light but without the harmful effects of UV exposure from sun bathing or using tanning beds. There are a number of topical products available that can be applied to the skin to help achieve that bronzed glow. Products are available for the face and body in the form of lotions, creams, gels, mousses and aerosol sprays, foams and wipes and they can be applied either at home or in a professional setting.

The main active ingredient in self-tanning products that is responsible for producing the temporary 'browning' or darkening of skin colour similar to a 'sun' tan is dihydroxyacetone (DHA) and has been used since the 1960's. DHA is a three carbon keto-sugar molecule derived from plants which, when applied to the skin, chemically reacts with the amino acids in the stratum corneum darkening the skin surface to produce the desired tanned appearance. This is known as the 'Maillard reaction'. Once applied to the skin, the chemical reaction is not immediate – it is usually visible within one to three hours after DHA application, but for maximal darkening it may require 8-24 hours and sometimes longer. Once the desired colour has developed, a fake tan resists being

washed off and will last between five to ten days, with the colour fading as skin cells desquamate. It is important to be aware that areas of the skin with a thicker epidermis, such as the elbows, knees, palms and soles, also stain more deeply, so care in application to these areas is recommended. It is recommended that skin is exfoliated prior to application to remove the build-up of dead skin cells, paying special attention to the knees, elbows and ankles. DHA does not stain the mucous membranes, but may stain the hair and nails.

The intensity of the skin colour achieved is dependent on the concentration of DHA in the formulation. Concentration of DHA varies from 2.5% to 10%, where the lower concentrations will provide a lighter tan and the higher concentration provides a greater darkening of the skin.

DHA is approved by the US Food and Drug Administration (FDA) only for topical application. It is listed in the regulations as a 'color additive for use in imparting color to the human body'. Its use in cosmetics – including sunless tanning" products – 'is restricted to external application, where 'externally applied' cosmetics are defined as 'applied only to external parts of the body'. However, DHA is not approved for the use on non-skin areas. It should not be inhaled, ingested or applied to the eyes, nose, lips or mucous membranes because the risks – if any, are not known. Based on the above, although freely available, it is not approved by the FDA for use in tanning spray booths.

Topically, DHA has a proven safety record with only a few reported cases of allergic contact dermatitis. It is always best that a 'use-test' is undertaken prior to use to ensure that there is no allergy to the product. Applying a small amount of product behind the ear or on the inner forearm and observing for signs of dermatitis (redness, oozing, itching, scaling) over the subsequent 24 hours is recommended.

There are several additional ingredients that are used in formulating self-tanning products. They include preservatives (eg MI – methylisothiazolinone), fragrances, thickening agents, emulsifiers, antioxidants and other additives. In the event of an irritant or allergic contact dermatitis, it is uncertain whether the DHA, other ingredient or combination of ingredients may have contributed to the adverse event. Patch testing performed by a Dermatologist is required to identify the nature of the dermatitic reaction and the exact ingredient(s) responsible.

Other adverse reactions that have been reported – primarily in the case of aerosol/spray tanning booths – include coughing, dizziness, and fainting. Some doctors have expressed some concern that chronic exposure to spray tanning products – especially in booths – may exacerbate asthma and predispose to pulmonary diseases such as chronic obstructive pulmonary disease (COPD). The risk of inhalation to both the consumer and personnel in a salon can be minimised by applying the product in a well-ventilated room, proper exhaust systems in professional settings, and protecting the eyes, nose and mouth with the use of a protective mask, nose plugs/filters, eye protection and lip protective balms.

The question of whether the possibility exists for the molecule to penetrate through the epidermis into the dermis leading to systemic absorption into the body and subsequent systemic toxicity has also been asked. There are reports that DHA induces DNA damage and free radical damage from UV radiation in-vitro, but statistically significant evidence is lacking in-vivo. Self-tanning lotions, sprays and creams generally only contain DHA at concentrations between 3-5%, levels considered at this time to be non-toxic and non-carcinogenic. Dermatologists agree that so long as they are used as directed (i.e. topically to non-compromised skin), there is no scientific evidence that sunless tanning products applied topically are harmful.

Other concerns that have been raised include the misconception that photo-protection is provided by a fake tan hence there is no need to apply a sunscreen.

Fake tans DO NOT offer any significant sun protective properties and are not meant to replace the use of a sunscreen. Some tanning products may contain an SPF, but the photo-protection is low and only lasts a couple of hours. In view of the fact that these products are usually applied the day before being outdoors, any minimal sun protection that may have been afforded would be long gone, so it is important that additional broad-spectrum sunscreen is applied every two to four hours when outdoors to provide sun protection from UV radiation – especially important during the summer months. A sunscreen can be applied over the top of a fake tan after it has been applied and allowed to dry.

Application of self-tanning products may cause certain pigmented lesions to darken in colour and change their appearance. Areas with hyperkeratosis (thicker epidermis) stain a darker colour. As a result, certain benign pigmented skin lesions such as seborrheic keratosis or actinic keratosis – will hyper-pigment, possibly leading to potential misdiagnosis of such lesions. It is advisable that anyone that may require or want a skin examination, should do so prior to using any sunless tanning products.

DHA is a popular, safe and reasonable alternative to a sun tan versus UV sun exposure. Whilst the only safe tan is indeed a fake tan, one should not forget that a fake tan should not be considered as providing any photo-protection from UV radiation whatsoever. Fake tans will not prevent sunburn from UV sun exposure nor should they be considered as an alternative to sunscreen use or following strict sun protective measures. Labels on such products should clearly provide this precaution to the consumer, and encourage that an appropriate sunscreen be applied in the usual fashion immediately prior any sun exposure. Anyone with sensitive or compromised skin should patch test prior to use and precautions taken when using aerosol forms of the product to prevent inhalation or ingestion.

formulation tips from A S Harrison & Co

This is the first in a series of articles that we like to call "Formulation Tips from A S Harrison & Co" where we highlight products selected by our team of formulating experts.

We will be focussing on Natural Hair Conditioning. There is an endless range of "natural" hair care products available today – all offering various features and benefits, but here are some ingredients that we think are worth considering.

These are the conditioning ingredients the team has selected:

- Pure Hyaluronic Acid – naturally derived and easier to incorporate into hair care formulations.
- AminoSensylTM HC – 100% natural COSMOS cationic emulsifier.
- Hydresia® SF2 – EcoCert oleosome emulsifier from Safflower.

We have all heard a lot about how beneficial Hyaluronic Acid is for our skin – let's share the joy with our hair!

Why try hyaluronic acid?

- Readily applicable for hair care formulae as they are available as a blend both with modified guar gum (cationHA-GTM), and Polyquaternium 10 (cationHATM-clear)
- Reside on hair even after rinsing (compared to HA alone)
- Perfect for scalp care formulas
- Improve barrier function of scalp
- Moisturise scalp
- Stabilise foam
- Phenomenal texture

Everyone in the office knows I love things that actually work. I was pondering our amazing Veegum versus HA for this article; I chose HA because yes, Veegum definitely works, but everyone knows it already! On the other hand, cationHATM is a new addition to our portfolio and it's a great opportunity to talk about it.

cationHATM is an innovative, amazing product which helps you incorporate all benefits of HA into your hair care product and be amazed by the difference in texture, foam and sensory.

For information on cationHATM and other innovative grades of Hyaluronic Acid, contact Maryam.

AminoSensylTM HC is a 100% natural, COSMOS cationic for hair conditioning and treatments. It the ultimate ingredient for the organic product marketplace.

Why AminoSensylTM HC will change your life:

- Your consumer will feel the luxury of a powerful cationic emulsifier, normally only available from synthetic products
- Can be used for elegant skin care products too
- Finally get to make palm-free & COSMOS hair products that work.
- Other cationics can be irritating or toxic, whereas AminoSensylTM is 100% safe & biodegradable.
- Designed and manufactured using the 12 Green Principles of Chemistry with an excellent sustainable story.
- AminoSensylTM HC also enables low viscosity sprayable emulsions and waterless sustainable formulations, like a Conditioner Bar.

For information on AminoSensylTM HC and the rest of the AminoSensyl range, contact Judy.

The reason for choosing Hydresia® SF2 for me was that it actually consists of natural Oleosomes from Safflower seeds. It is something that I know is 100% natural meaning that it is found in nature as Oleosomes. I wasn't aware of what Oleosomes were but at A S Harrison & Co, I have learnt that these structures are found naturally in plant seeds that contain oils. These Oleosomes are what the seed uses as energy until germination.

The other unique benefit is that Oleosomes can also act as nature's own emulsification system – it acts as a powerful emulsifier and you can make stable formulations with high oil content.

Hydresia® SF2 consists of 65% dispersions of Oleosomes in water and due to its unique structure, it can act as a cold-process natural emulsifying system, or even be used as a conditioning additive in shampoos.

It is also ECOCERT Natural certified so it can safely be used in "natural formulations."

Having personally worked in manufacturing, developing new products, I also understand the benefits of having an ingredient that can be multifunctional in order to reduce inventory and costs. Hydresia® SF2 is a perfect multifunctional ingredient that can be used in haircare, skincare and even alcohol hand sanitisers. It is also known to function as Mother Nature's delivery system and provides SPF boosting properties in sunscreens.

For more information on Hydresia® SF2, contact Annis.

These are unique ingredients that can be used in natural hair conditioners with proven efficacy and performance. We will continue to share our "favourite" ingredients in our next articles. Stay tuned.

A S Harrison & Co has an extensive range of natural ingredients that will add value to your formulations and your customers.

For more information and samples please contact your A S Harrison & Co account manager or email performanceingredients.ash@harrison.com.au or call us on +61 (0)2 8978 1016

sunscreen highlights

by John Staton

Baby, please pass the sunscreen

There are many sunscreens offered in the marketplace as specifically targeted for babies and young children. However, it would appear that many do not clearly identify the age group of "baby", "toddler", "junior", or "kids".

Defining a Baby

According to typical dictionary definitions, a baby is an extremely small child. No definition appear to give a cut-off age but typically for therapeutics the first age break point appears at 6 months.

There is apparently some confusion as to the cut off age for use of sunscreen by smaller children – 6 months and below. Additionally, it would appear that label instructions for some products which are targeted for baby include a cautionary statement along the lines of seeking medical advice before applying to children under 6 months.

A quick review of TGA Listed sunscreens reveals that many are SPF 50 or 50+, the highest category of protection. These contain a combination of organic actives. Several SPF 30 baby products appear and these contain Zinc oxide alone.

What is the right SPF for a baby formula? Should they be especially formulated?

Formulating specifically for babies and toddlers should imply attention to specific low irritancy potential, but it would appear that, at least as far as actives are concerned, there can be no difference between an adult and a baby formulation. In my experience, the situation can often be that the product is developed for adult use and then tested for dermal safety and rebadged. Yes, sensitive panel HRIPT can support a general suitable claim but, as we do not test on babies, there is always the potential for an issue to arise post product launch. Such a situation occurred only recently and potentially the issue appeared to be with an excipient in this case.

"Hypoallergenic" is also claimed on several baby sunscreen products, but required support for this claim is ill-defined in Australia and unclear even in the E.U. (1)

Overall, there appears to be no clear guidance on how a product for sun protection of babies or young children should be specifically formulated and, beyond SPF testing, how its efficacy should be certified.

The Australasian College of Dermatologists current advice (2) is "For babies less than 6 months of age, the widespread use of chemical sunscreens is not recommended. Sun protection is best provided with shade, clothing, hats and physical sunscreen and by avoiding prolonged outdoor exposure during the middle of the day. "

Cancer Council Australia National Council Control Policy Control (3) states "It is recommended that babies under 12 months are kept away from direct sunlight when UV levels reach 3 or above."

From these perspectives, the question arises as to whether it is of the appropriate to use of sunscreens on babies as a routine procedure.

A 2017 article by Choice (4) identified that "Some had been told never to use it on babies under six months, others had been told it was OK in small doses, while others still had not been told anything at all and were under the impression it was fine to use early on."

References

1. The Australasian College of Dermatologists Position Statement Sun protection and sunscreens Feb 2017.

2. Cancer Council Australia's National Skin Cancer Committee Fact sheet – Sun protection and babies (0-12 months) Updated Dec 2017

3. Technical document on cosmetic claims Agreed by the Sub-Working Group on Claims 3 July 2017

4. Is sunscreen safe for young babies? 9th Feb 2017.

NEW DIRECTIONS | AUSTRALIA

2020 Education / Workshops New year and new beginnings for Target audiences to research about manufacturing services and ingredient purchases for their beauty journey. NDA workshops start from late Feb, so aligning ad with that. Upcoming workshops and the benefits it will give the customers business journey. - Free help desk / technical support. Where will your business take you in 2020?

In life, there are some things that are best experienced first-hand, that no computer screen, glossy photograph or book can truly capture. When it comes to cosmetic science, there is something magical about rolling up the sleeves and getting stuck in...

From the quiet crack the tamper-evident seal makes when opening a fresh bottle of essential oil to the glug, glug, glug of liquid extract into the laboratory glass beaker you just know you are in for a feast of the senses and a massage of the brain!

This opportunity to play, to feel your way through each deliberate step whether it be measuring or sploshing, stirring or pouring makes the resulting serum, night cream, mask or spritzer feel all-the-more exciting. Knowing you are being mentored and encouraged by industry experts focused on teaching in a way that explains and shows how, rather than what to think gives you the confidence to explore your own creative potential!

All this and more happens at New Directions.

Located in the heart of Sydney's inner west, this bustling cosmetic manufacturer hosts an exciting range of workshops above the factory in the beautiful multi-sensory ambience of the showroom. Meanwhile downstairs cosmetic products are created, manufactured, packed and despatched to brand owners and businesses all over the world.

Over the last fifteen years of teaching, New Directions has provided the space, industry know-how and encouragement to many a budding entrepreneur and beauty product lover as they turned their dreams into reality. Why not take your career in a New Direction this year...

Our current workshop schedule is available online. For bookings and enquiries contact Marly Sparta 02 8577 5914.

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nda@newdirections.com.au | **newdirections.com.au** | @newdirectionsaus

SCST to host 2021 ASCS Conference

by Pam Jones

Welcome to the New Year of the Rat. Our first edition for 2020 looks at the small island of Taiwan. This small country has a population of app 24.5m. It is a highly developed country with a high level of education.

The Society of Cosmetic Scientists of Taiwan (SCST) has a membership of app 100. Each year the members participate in activities such as the IFSCC Conference and the ASCS Conference. They also participate in an exchange program; their last exchange was with the Japanese Society. SCST work very closely with Providence University in Taichung City.

During 26th-28th April 2021, the society is to host the 2021 ASCS Conference. This conference to be held at the Hotel National in Taichung City. You should add this in your diary. A visit to Taiwan to understand the technology in Personal Care is a must-do.

The President of the Society is Professor Lin, Chih-Chien, and the Secretary-General is Dr Chang, Nai-Fang. E-MAIL: scsttaiwan@gmail.com

Market Summary

Currently, in Taiwan, a HOT new product is ampoule packs. They originated in the pharmaceutical sector; however, the latest products launched into the personal care market imply professional, premium, drug-like efficacy in minimal pack size. These new launches are for anti-ageing ampoules as well as intense hydration.

Other product trends that are popular in the market are

- hydration using hyaluronic acid
- anti-aging using peptides/ polypeptides
- natural elements/ herb extracts
- lightening using high dosage of Vitamin C

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HOT PRODUCTS (locally produced)

DR. WU Intensive Renewal Serum with Mandelic Acid 18%

Dr Satin Caviar GHK-Cu Miracle Protection Spray

For Beloved One, Watson's best-selling facial mask in 2019.

Neogence Hydrating Water with Glacier Water

TTM Hydration Regenerating Skin Boosting Mask

Facial masks are trendy and said to be worth USD 64M in retail value yearly. The various types available are essential hydration, wrinkle reduction, elasticity improvement and skin brightening.

Popular mask brands are sold by Watsons. Another well-known brand is the Japanese brand Matsumoto.

Sales channels are also showing a trending change. Purchasing of "drug

LITS Anti-aging Moisturization Repair Mask, Matsumoto's best-selling facial mask in 2019.

store" products were originally for cosmeceuticals, but these products are now via the general Personal Care market channel. Two significant rivals were Watsons and Cosmed, but far more players are joining into the competition, e.g., Poya.

K-beauty with a lot of celebrity promotion also influences the Taiwan market and customers' purchasing intentions.

Dominating the mass markets are MNC like Unilever, P&G, Kao and a local company Nice.

Besides TV commercials, promotion for local brands is also via on-line shopping with blogger and YouTuber's demonstrations.

The manufacturing sector has changed over the past years in Taiwan. Once there were many MNC's. Shiseido had three manufacturing sites in Taiwan; they now have one location in Hsinchu. Shiseido has moved most other manufacturing to mainland China.

Today the significant manufacturers in Taiwan are OEM/ODM* working with marketing companies; these plants number more than 200 local manufacturers.

Below I have listed some OEM/ODM manufacturers as well as their web sites. They are available in English, and it is an opportunity for you to see just how technically advanced Taiwan Personal Care Industry is.

In the Taipei area, Stella Beauty is a well-known manufacturer.

http://www.stellarbeauty.com.tw/index.asp

In Southern Taiwan, Colame is one of the well-established OEM/ODM players, they have also set up their micro lab to conduct challenge testing.

http://www.colame.com.tw/index.php

In central Taiwan, Maxgut Biochemical is well known for its tooling works for facial masks.

https://www.maxgut.com/tw

*Original Equipment Manufacture/ Original Design Manufacture

My thanks to Dr Kevin Chang for his input into this article.

healing benefits of clay

in at-home, eco-friendly solution for multiple ethnicities

Obtained from a microorganism isolated from clay, new Lipotec™'s Uniclay™ biotech ingredient mimics the multiple beneficial effects of clay on the skin, helping create cleaner, smoother and healthier skin in different ethnicities.

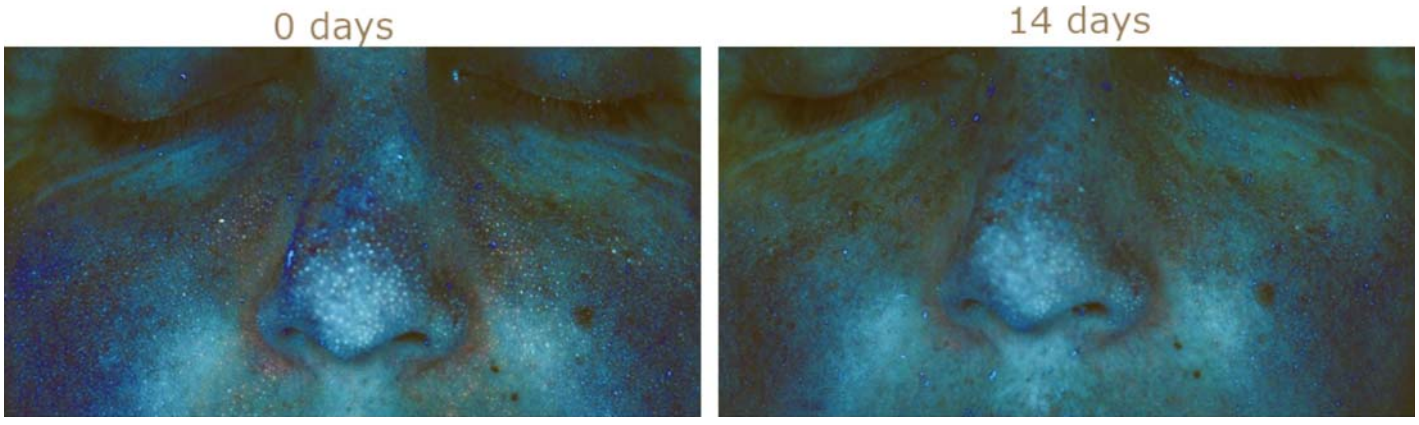
Clay is one of the oldest skin care treatments still used today, thanks to its many cosmetic and wellness benefits, which include impurity removal, smoother skin, improved cellular metabolism and reduction of oxidative stress and inflammation. The results of clay treatments can also lead to enhanced emotional well-being and self-perception.

Sustainably sourced near a wetland in Catalonia, Spain, Uniclay™ biotech ingredient has shown multiple benefits in in vivo and in vitro efficacy studies. In vivo a 6.6% decrease in red spots was observed after 28 days of treatment at 2%. In only 14 days of applying a cream containing 3% ingredient an increase by 9.5% and 13.5% in smoothness and softness respectively was shown, as well as a 50.7% reduction in porphyrins, for enhanced skin purification.

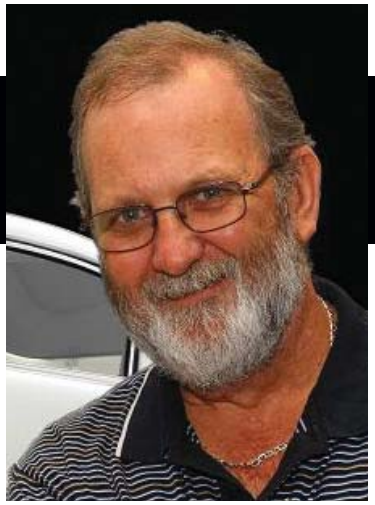
A third clinical test was carried out using the Mirror Test™ technique, with participants answering questions about their appearance while standing in front of a mirror at the beginning and end of the study. Responses were evaluated for vocal intensity and tone, demonstrating stress level about their reflection, and words used to describe their appearance. Compared to results with a benchmark clay mask, enhancement of well-being and self-perception was similar when using the Uniclay™ biotech ingredient.

Based on these studies, incorporating Uniclay™ biotech ingredient into skin care creams for face and body provides consumers with an ideal solution for not only enhancing the skin of all types of ethnicities, but also in boosting well-being and self-perception in an eco-friendly way.

For more information, please contact Robert McPherson, Account Manager for Australia and New Zealand, at Robert.McPherson@Lubrizol.com or Tel: +61 (02) 9741 5237



Skin purifying effect (reduction in porphyrins)



Part 50 –

by Ric Williams

Muds and Clays

Ancient civilizations used cosmetics for many reasons: for rituals, war paint, beauty, and health. Egyptians (both male and female) apart from oils and herbal extracts used masks, and makeup for beauty, and health. Greeks used red clays for lipstick and chalk to whiten and protect their faces. Romans, well-to-do citizens traveled to resorts for treatments similar to what can be found in modern spas, including yoghurt as a moisturiser, bathing in milk and mud baths. Mud or clay masks are used to detoxify, deeply cleanse, and soothe skin.

Many of the ingredients in these cosmetics came directly from the earth, such as clay, mud, minerals, and some pigments.

Muds

Definition of Mud (Merriam-Webster Dictionary)

"A slimy sticky mixture of solid material with a liquid and especially water, especially: soft wet earth"

Definition of Mud (Wikipedia)

Mud is a liquid or semi-liquid mixture of water and any combination of different kinds of soil (loam, silt, and clay). It usually forms after rainfall or near water sources. Ancient mud deposits harden over geological time to form sedimentary rock such as shale or mudstone (generally called lutites). When geological deposits of mud are formed in estuaries, the resultant layers are termed bay muds.

Mud Masks

Often the terms "mud mask" and "clay mask" are used interchangeably, although mud masks usually have a more moisturizing formula, along with other raw materials taken from the earth like silt or peat. Clay masks are mostly used for clarifying, mattifying and concealing.

Dead Sea Mineral Mud

The Dead Sea is a salt lake bordered by Jordan to the east and Israel and the West Bank to the west. It lies in the Jordan Rift Valley, and its main tributary is the Jordan River. Its surface and shores are 430.5 meters below sea level, Earth's lowest elevation on land. Dead Sea Mud is a natural mineral-rich product (silt) that is harvested from the shores of the Dead Sea.

The mud is a fine grained, smooth viscous dark brown to black paste free from grit and sand, with a natural characteristic smell, a pH ranging from 7.5 – 8.9 and a specific gravity ranging from 2.257 to 2.386. Dead Sea Mineral Mud contains high levels of sodium, potassium, calcium, magnesium, chlorides, bromides and sulphates. The variation in Dead Sea mud properties depends on the location with reported differences between the eastern and western sides of the Dead Sea being considerable, the eastern side source being preferred.

Ric Williams B.Sc. Dip.Env St.
Cosmepeutics International
This column is intended not only as an education tool for non-technical people or beginners in our industry, but as a forum for those wishing to enlighten all about recent technology advances and new ideas. I hope experienced scientists will also contribute to this ideal and if you wish to do so please email me at: ric@cosmepeutics.net.au and I will publish your comments.

Dead Sea mud, probably the most famous mud used in cosmetics, helps maintain skin's moisture level and hydration and is used for its extreme effectiveness in improving the texture of the skin (including wrinkles), hair and scalp, reportedly helping alleviate aches in muscles and joints and has been as an effective treatment of acne. It can also extract toxins present on skin, by absorbing them into the mineral matrix, which is then washed away (ie via a mud mask).

Simple Marine Dead Sea Mud Mask

Purified Water	7.00%
Carageenan (Thickener)	1.00%
Glycerine	10.00%
Preservative	1.00%
Dead Sea Mud	50.00%
Marine Collagen	1.00%

Volcanic Ash

Volcanic ash has been used for centuries by the native Pacific Islanders for its cleansing qualities. Volcanic ash consists of fragments of rock. minerals and volcanic glass, created during volcanic eruptions and measuring less than 2 mm in diameter and are finely crushed to as small as 1/1,000th of an inch (or 0.025mm). The ash is insoluble in water and is often used as a mild exfoliant.

Clays (Minerals)

Definition of Clay (Merriam-Webster Dictionary)

"A soil that contains a high percentage of fine particles and colloidal substance and becomes sticky when wet"

Definition of Clay (Wikipedia)

Clay is a finely-grained natural rock or soil material that combines one or more clay minerals with possible traces of quartz (SiO2), metal oxides (Al2O3, MgO etc.) and organic matter. Geologic clay deposits are mostly composed of phyllosilicate minerals containing variable amounts of water trapped in the mineral structure. Clays are plastic due to particle size and geometry as well as water content, and become hard, brittle and non–plastic upon drying or firing. Depending on the soil's content in which it is found, clay can appear in various colours from white to dull grey or brown to deep orange-red.

Although many naturally occurring deposits include both silts and clay, clays are distinguished from other fine-grained soils by differences in size and mineralogy. Silts, which are fine-grained soils that do not include clay minerals, tend to have larger particle sizes than clays. There is, however, some overlap in particle size and other physical properties. The distinction between silt and clay varies by discipline. Geologists and soil scientists usually consider the separation to

occur at a particle size of 2 μm (clays being finer than silts), sedimentologists often use 4–5 μm, and colloid chemists use 1 μm. Geotechnical engineers distinguish between silts and clays based on the plasticity properties of the soil, as measured by the soils' Atterberg limits. ISO 14688 grades clay particles as being smaller than 2 μm and silt particles as being larger.

Kaolin (Hydrated Aluminium Magnesium Silicate)

Kaolin, also called china clay (synonyms: china clay, nacrite, kaolinite, calcined kaolin, metakaolin), is a soft white clay that is an essential ingredient in the manufacture of china and porcelain and is widely used in the making of cosmetic products. Kaolin is named after the hill in China (Kao-ling) from which it was mined for centuries.

The commonly used USP grade, is a pure natural mineral (anhydrous aluminum silicate) composed of kaolinite with low iron content. It consists of 44-46% silicon dioxide, 37-40% aluminum oxide, 0.2-0.4% magnesium oxide, 0.03-0.06% calcium oxide, 0.2-0.4% ferric oxide, and 1.5-2.0% titanium dioxide. pH 5 (5% solution). The average particle size 1.0-1.4 micrometers and is an off-white fine powder, odorless and insoluble in water. When kaolin is mixed with water in the range of 20 to 35 percent, it becomes plastic. It is used as an absorbent for skin borne oil and contaminants (eg. in face masks) and as a concealer ingredient due to its clean white mattifying appearance).

Montmorillonite Clay (hydrated Sodium Calcium Aluminium Magnesium Silicate Hydroxide (Na,Ca)0.33(Al,Mg)2(Si4O10)(OH)2·nH2O). Potassium, Iron, and other cations are common substitutes giving the clay many colour variations. eg

Australian Clays – Beige, Ivory, Olive Green Pink, Red, Yellow

Brazilian Clays – Black, Cocoa Brown, Gold, Grey, Purple, Red, White

French Argile Clay – Green, Pink, Red, White, Yellow

French Clay – Green, Nude, Pink, Red, White, Yellow

Used as an absorbent for skin borne oil eg. in face masks, and a base for foundations.

Bentonite

Bentonite (/ˈbɛntənʌɪt/)[1] is an absorbent aluminium phyllosilicate clay consisting mostly of montmorillonite.

The different types of bentonite are each named after the respective dominant element, such as potassium (K), sodium (Na), calcium (Ca), and aluminium (Al). Bentonite usually forms from weathering of volcanic ash, most often in the presence of water.

Benefits

Used as thickener, absorbent, filler, texturizer and binder in various skin and hair care products and color cosmetics

Has thickening and suspending properties (e.g suspends

pigments) and effectively thickens and stabilizes emulsions

Acts synergistically with common thickeners like xanthan gum and HE cellulose

Simple Cream Mask #1

Purified Water	30.00%
Sodium Phytate	0.10%
Triethanolamine 99%	0.20%
Stearic Acid	4.00%
Cetyl Alcohol	3.00%
Polysorbate 60	5.00%
Purified Water	7.20%
Australian Clay – Ivory	15.00%
Titanium Dioxide	3.00%
Bentonite	13.50%
Glycerin	10.00%
PEG-7 Glyceryl Cocoate	8.00%
Preservative	1.00%

Simple Cream Mask #2

Deionised Water	36.40%
Sodium Phytate	0.10%
Glycerine	2.00%
Aloe Vera Juice	4.00%
Sunflower Oil	4.50%
Macadamia Nut Oil	0.50%
Beeswax	4.50%
Shea Butter	0.50%
Cetomacrogol 1000	0.50%
Emulsifying Wax	3.00%
Glyceryl Stearate	3.00%
Preservative	1.00%
Australian Clay – Ivory	40.00%

Simple Marine Clay Mask

Purified Water	47.00%
Carageenan (Thickener)	1.00%
Glycerine	10.00%
Preservative	1.00%
Australian Clay – Ivory	40.00%
Marine Collagen	1.00%

Laponite (Sodium Magnesium Silicate)

A synthetic smectic clay that forms a clear, thixotropic gel when dispersed in water, hence is used as a thickener. It forms lamellar structures in water that allow ease of movement (spreading) but quickly reform into thick suspensions when the force is reduced.

Zeolite

Zeolites are microporous, aluminosilicate minerals commonly used as commercial adsorbents and catalysts.[1] The term zeolite was originally coined in 1756 by Swedish mineralogist Axel Fredrik Cronstedt, who observed that rapidly heating the material, believed to have been stilbite, produced large amounts of steam from water that had been adsorbed by the material. Based on this, he called the material zeolite, from the Greek ζεω (zéo¯), meaning "to boil" and λι`θος (líthos), meaning "stone".[2] The classic reference for the field has been Breck's book Zeolite Molecular Sieves: Structure, Chemistry, And Use.[3]

Zeolites occur naturally but are also produced industrially on a large scale. As of December 2018, 245 unique zeolite frameworks have been identified, and over 40 naturally occurring zeolite frameworks are known.[4][5] Every new zeolite structure that is obtained is examined by the International Zeolite Association Structure Commission and receives a three letter designation.[6]

Zeolites have a porous structure that can accommodate a wide variety of cations, such as Na+, K+, Ca2+, Mg2+ and others. These positive ions are rather loosely held and can readily be exchanged for others in a contact solution. Some of the more common mineral zeolites are analcime, chabazite, clinoptilolite, heulandite, natrolite, phillipsite, and stilbite. An example of the mineral formula of a zeolite is: Na-2Al-2Si-3O-10·2H2O, the formula for natrolite. These cation exchanged zeolites possess different acidity and catalyse several acid catalysis.[7][8][9]

Natural zeolites form where volcanic rocks and ash layers react with alkaline groundwater. Zeolites also crystallize in post-depositional environments over periods ranging from thousands to millions of years in shallow marine basins. Naturally occurring zeolites are rarely pure and are contaminated to varying degrees by other minerals, metals, quartz, or other zeolites. For this reason, naturally occurring zeolites are excluded from many important commercial applications where uniformity and purity are essential.

Zeolites are the aluminosilicate({\displaystyle {\ce {AlO4^5-}}}{\displaystyle {\ce {AlO4^5-}}} and {\displaystyle {\ce {SiO4^4-}}}{\displaystyle {\ce {SiO4^4-}}}) members of the family of microporous solids known as "molecular sieves", and mainly consist of Si, Al, O, and metals including Ti, Sn, Zn, and so on. The term molecular sieve refers to a particular property of these materials, i.e., the ability to selectively sort molecules based primarily on a size exclusion process. This is due to a very regular pore structure of molecular dimensions. The maximum size of the molecular or ionic species that can enter the pores of a zeolite is controlled by the dimensions of the channels. These are conventionally defined by the ring size of the aperture, where, for example, the term "8-ring" refers to a closed loop that is built from eight tetrahedrally coordinated silicon (or aluminium) atoms and 8 oxygen atoms. These rings are not always perfectly symmetrical due to a variety of causes, including strain induced by the bonding between units that are needed to produce the overall structure, or coordination of some

of the oxygen atoms of the rings to cations within the structure. Therefore, the pores in many zeolites are not cylindrical.

Simple Zeolite Mask

Water	59.20%
Zeolite Fine Powder	22.50%
Vitus vinnefera (Grape) Seed Oil	4.50%
Macadamia Integrifolia (Macadamia) Seed Oil	0.50%
Beeswax	1.50%
Butyrospermum parkii [Karite/Shea] Seed Butter	2.50%
Cetearyl Glucoside (and) Cetearyl Alcohol	4.00%
Glyceryl Stearate	1.50%
Ethylene Glycol Stearate	1.00%
Aloe barbadensis (Aloe vera) Concentrated Powder	0.10%
Ethanol	2.60%
Essential Oils	0.10%

Alum (**Potassium Alum** – Aluminium Potassium Sulfate)

Used as an astringent (in toners or insect bite relief products) or in face masks for its drying and refining effect.

Alum (**Ferric Alum** – Aluminium Iron Sulfate)

Talc (Hydrous Magnesium Aluminium Silicate)

Used as an absorbent for skin borne oil eg. in body talcs.

There has been recent bad press about using talc and this has been associated with the use of talc powder over long term application. This bad press is the assertion that impure grades of talc contained asbestos.

The use of purified talc in liquid and cream formulations should not have this problem, hence could still be safely used in foundations, compacts, rouge and other facial cosmetics.

Liquid Foundation base

Purified Water	60.00%
Magnesium Aluminum Silicate (Veegum)	0.25%
Xanthan Gum	0.25%
Carboxymethyl Cellulose Gum	0.25%
Sodium Phytate	0.10%
Triethanolamine 99%	1.00%
Steareth-21	0.70%
Glycerine	4.30%
Titanium Dioxide Anatase	5.40%
Colour Grind in Castor Oil	q.s
Talc Sterilised	5.00%
Kaolin BP	2.50%
Stearic Acid Veg	2.50%
Isostearic Acid	2.00%
Stearyl Dimethicone	4.00%
Hydrogenated Polydecene	2.00%
Steareth-2	0.30%
Jojoba Oil	2.50%
Vegetable Oils	5.50%
Preservative	1.00%
Frzagrance	0.10%
Purified Water	q.s.

Titanium Dioxide (TiO2)

Mostly used as a sunscreen (particularly the microfine grades) although used to "whiten" thin emulsions.

Zinc Oxide (ZnO)

Mostly used as a sunscreen (particularly the microfine grades) although also used as a skin protectant (eg in baby creams or Zinc and Castor Oil creams) or in face masks.

Silica (Sand or SiO2)

Coarse grades (sands) – Used as abrasives in skin peels and toothpastes.

Fine grades (Amorphous Silicas) – Used as thickeners in cosmetics and toothpastes.

Others

Carbon Black – Activated Charcoal

Carbon Black (Common charcoal) can be made from peat, coal, wood, coconut shell, or petroleum by burning the organic source leaving the charred remains. "Activated charcoal" is made by heating Carbon Black in the presence of a gas. This develops lots of internal spaces or "pores", that allow the activated charcoal "trap" other materials such as oils, heavy metals and contaminants.

A simple **Activated Charcoal Mask** formula is

Aloe vera Juice	87.50
Bentonite	11.00
Preservative	1.00
Activated Charcoal	0.50
Essential Oils	Trace

Calamine (Zinc Oxide (astringent) with 0.5% Ferric (Iron) Oxide (antipruritic))

Calamine, also known as calamine lotion, is a medication used to treat mild itchiness. This includes from sunburn, insect bites, poison ivy, poison oak, or other mild skin conditions. It may also help dry out skin irritation. It is applied on the skin as a cream or lotion.

Calamine Lotion BP

Actives	Calamine	15.0% w/v
Zinc Oxide		5.0% w/v
Excipients	Purified Water (Carrier)	
	Bentonite (thickener/stabilizer)	
	Sodium Citrate (stabilizer)	
	Glycerol (Humectant)	
	Phenol (Preservative)	

Sulfur (S)

Elemental Sulfur (a pale yellow crystalline powder) is used as an antiseptic and a scabicide for seborrhoic skin.

Final Word

In my view "Mineral Makeup and Mineral Cosmetics" appear to be a range of cosmetic products that contain some of the minerals quoted above (and not usually at significant levels), but are usually based on common cosmetic bases using common organic or synthetic components such as humectants, emulsifiers, oil phase and actives. They are not really different to what we have been using in the cosmetic industry, for many years, as Foundations, Compacts, etc. but have been marketed with a new twist for an old, tired, format.

Stabilizing Skin Endogenous Photosensitizers:

The Ultimate Solution for Total Photoaging Prevention

by Robert S. Hu (Presenter) and Eileen Zhang

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Abstract

A family of compounds structurally consisting of fused aromatic rings conjugated with cyanoacrylates (Fused Ring Conjugated Cyanoacrylates: FRCC) were designed and synthesized. These compounds, with no absorption in the visible light wavelength range (>400 nm), were extremely effective in quenching excited states of various endogenous photosensitizers commonly found in the skin via an excited state electron transfer mechanism. This unique technology enables total elimination of reactive oxygen species derived from the excitation of the endogenous photosensitizers by UV or visible light energy, thus thoroughly preventing photoaging. This technology extends photoaging prevention into the visible light domain. With no absorption in the visible light region by FRCC, they are uniquely suitable for cosmetic applications for preventing UV-Visible induced photoaging where a colorless formulation is critical for acceptable consumer perception. Compared to the reactive nature of most antioxidants, FRCC compounds are stable physically, chemically, and photochemically so that they are safe and effective in various cosmetic applications.

Introduction

Reactive oxygen species (ROS) generated by the action of sunlight on endogenous chromophores in human skin are believed to play a significant role in skin damage and disease, including premature skin aging and cancer.[1] An important class of endogenous chromophores are porphyrins which are distributed throughout the plant and animal kingdoms where they play essential roles in biological processes including oxygen transport and storage (heme), electron transport (cytochromes), and energy conversion (chlorophylls). Protoporphyrin IX (PpIX) is the direct chemical precursor to heme, a universal component of human tissue. Detailed photophysical studies on PpIX have been reported.[2-4] PpIX absorbs UV and visible light below 640 nm and shows fluorescence with peaks at 630 and 698 nm with a lifetime of 19.2 ns in acetonitrile solution. PpIX singlet excited states intersystem cross into triplet states with high quantum yields (f = 0.63).[5] These long-lived triplet states are quenched efficiently by molecular oxygen to generate singlet oxygen ($^1O_2$),[6, 7] a highly reactive form of molecular oxygen that reacts with cellular components to cause protein oxidation, lipid peroxidation, and DNA damage.[8, 9] In addition, $^1O_2$ is the precursor of other reactive oxygen species (ROS), such as peroxide radicals, superoxide and hydroxy radicals. Increased ROS level generate a series of signal transduction pathways by activation of skin cell surface receptors, including receptors for epidermal growth factor, interleukin-1, insulin, keratinocyte growth factor and tumor necrosis factor-a. Activated cell surface receptors further result in up-regulation of the expression and functional activation of the nuclear transcription factor, which results in reduced collagen gene transcription and degradation of collagenous and noncollagenous molecules in the extracellular matrix, impairing the structural integrity of the skin.[10] Therefore, controlling the generation of ROS is an important strategy for skin anti-aging technology and prevention of skin cancer. Herein we report our surprising finding that fused ring cyanoacrylate derivatives (FRCC) 2-6 (Chart 1) have the ability to quench the excited states (singlet and triplet) of the endogenous chromophore PpIX and by doing so significantly reduce its photo-generation of singlet oxygen. Under the same condition, the non-

fused ring derivative 1, Chart 1, did not quench excited states of PpIX.

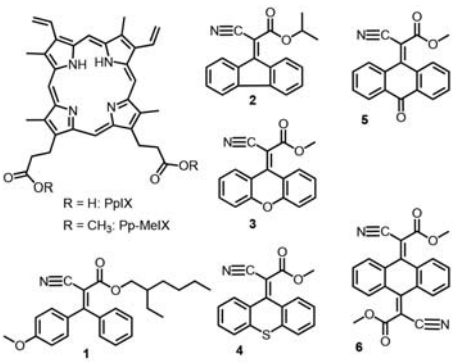

Chart 1 Structures of protoporphyrin IX (PpIX) and representative compounds studied (1-6).

Results and Discussion

Singlet-excited state quenching of PpIX by 1-6 was studied by time-resolved fluorescence quenching. PpIX fluorescence decay traces were recorded at 690 nm after pulsed excitation at 496 nm in the absence and presence of various concentrations of 1-6. The fluorescence lifetimes of PpIX were determined at each quencher concentration. The bimolecular quenching rate constants kqS were determined from the slope of the plots of the inverse fluorescence lifetimes vs. the quencher concentrations (Fig. 1). High quenching rate constants of ~ 4-5´ 109 M-1s-1 were found for 2, 3, 5, and 6. The sulfur bridged FRCC 4 showed a one order of magnitude lower quenching rate constant. The non-bridged control compound 1 did not show any observable PpIX singlet excited state quenching.

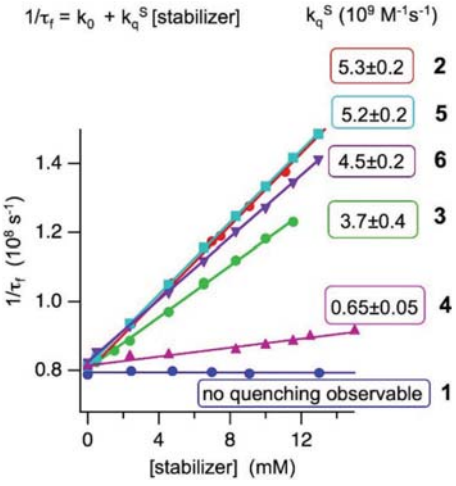

Fig. 1 Determination of the bimolecular quenching rate constants kqS of quenching of PpIX fluorescence by compounds 1-6 from the slope of the plot of the inverse fluorescence lifetime vs. the quencher concentration. The excitation wavelength lex = 496 nm.

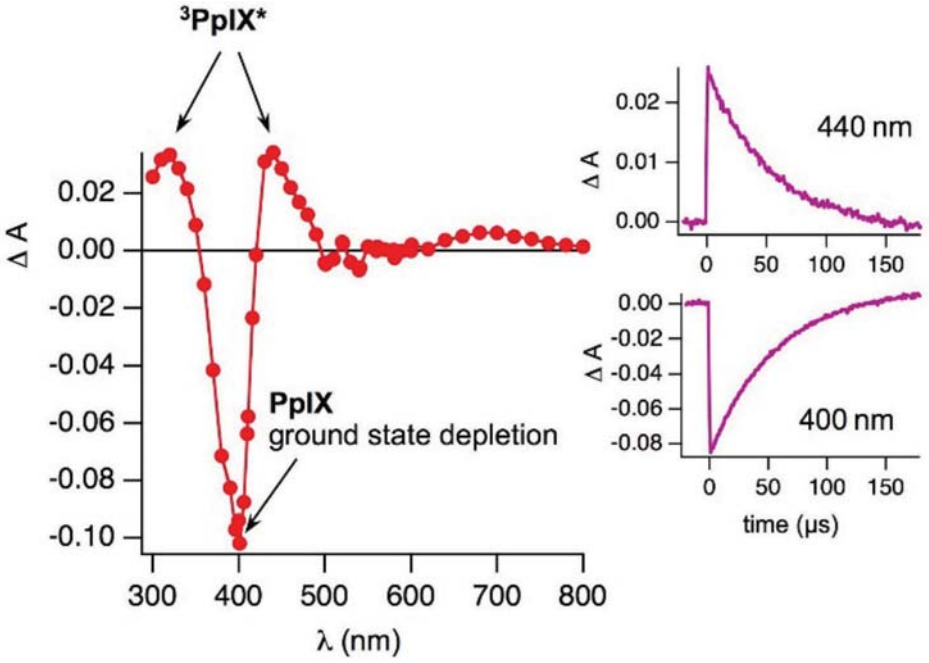

Fig. 2 Transient absorption spectrum of an argon saturated acetonitrile solution of PpIX recorded 0.1 to 1.5 μs after pulsed laser excitation (532 nm, 7 ns pulse width). Kinetic traces monitored at 400 nm and 440 nm are shown on the right.

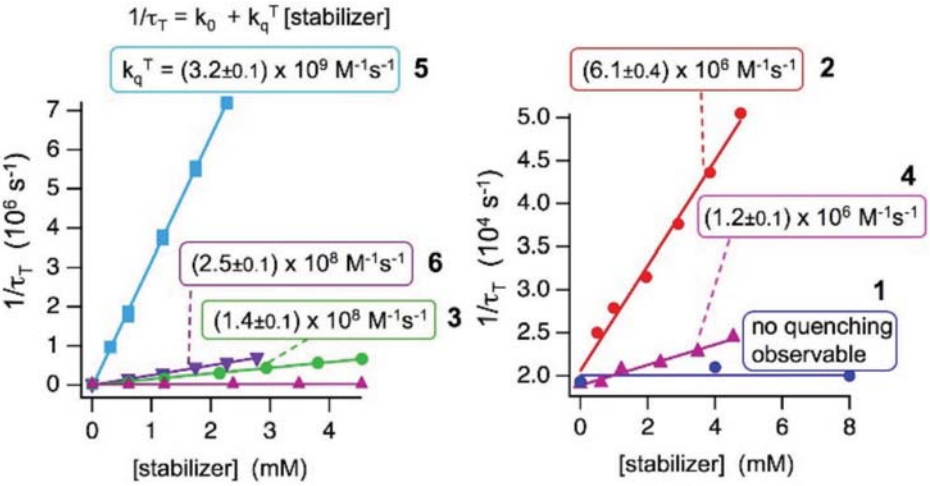

Fig. 3 Determination of the bimolecular quenching rate constants kqT of quenching of PpIX triplet states by 1-6 from the slope of the plot of the inverse triplet lifetime (monitored at 440 nm) vs. the quencher concentration.

Because of the longer lifetime of PpIX triplet states compared to singlet excited states, reactions and phototoxicity are more likely to occur from triplet states. The kinetics of PpIX triplet state reactivity can be followed by laser flash photolysis. Fig. 2 shows the transient absorption spectrum of PpIX in acetonitrile solutions after pulsed laser excitation. At 400 nm the ground state bleaching of PpIX is observable. The absorbance at 440 nm was assigned to the triplet state.[11] In deoxygenated acetonitrile solutions, the triplet decayed with a lifetime of 52 μs with simultaneous recovery of the ground state absorption (Fig. 2, insets).

In the presence of the quenchers FRCC 2-6 the triplet lifetime decreased and the ground state absorption, monitored at 400 nm, was recovered. From the plot of the inverse triplet lifetimes vs. the quencher concentration the bimolecular triplet quenching rate constants kqT were determined (Fig. 3).

Table 1 summarizes the measured quenching rate constants. Although for PpIX singlet excited state quenching, the rate constants for FRCC 2, 3, 5 and 6 are similar (kqS ~ 4-5´ 109 M-1s-1), the rate constants for triplet state quenching cover a range over several orders of magnitude. The highest rate constant was observed for 5 (kqT = 3.2´ 109 M-1s-1) whereas 2 shows a rate constant of only 6.1´ 106 M-1s-1. Consistent with

the singlet excited state quenching, the sulfur bridged FRCC 4 shows an even lower triplet quenching rate constant (kqT = 1.2´ 106 M-1s-1) and the non-bridged control compound 1 did not quench PpIX triplet states.

Compound studied	k_q^S (10^9 $M^{-1}s^{-1}$)	k_q^T (10^9 $M^{-1}s^{-1}$)	K_{ST} (M^{-1})
1	-[a]	-[a]	0.2
2	5.3	0.0061	30
3	3.7	0.14	27
4	0.65	0.0012	1.2
5	5.2	3.2	240
6	4.5	0.25	31

[a] no quenching observable

Table 1 Rate constants of quenching of singlet and triplet excited states of PpIX (kqS and kqt, respectively) by compounds 1-6. Stern-Volmer constant (KST) of suppression of 1O2 phosphorescence by compounds 1-6.

Singlet oxygen phosphorescence measurements were performed to investigate if the large differences in triplet quenching rate constants can be reflected on the singlet oxygen yields generated from PpIX photoexcited states. Singlet oxygen, the first excited state of molecular oxygen, shows a characteristic phosphorescence at 1270 nm.[12] The dimethyl ester derivative of PpIX (Pp-MeIX, Chart 1) was selected as sensitizer because of its good solubility in a solvent with long singlet oxygen lifetime (CDCl3). Longer singlet oxygen lifetime helps in improve the detection and accuracy. Singlet oxygen lifetimes up to 8.9 ms have been reported in CDCl3.[13] The excited state properties of protoporphyrin IX are not affected significantly by the methyl ester functionality. Air saturated CDCl3 solutions of Pp-MeIX were irradiated with a pulsed Nd-YAG laser with visible light at 532 nm, a wavelength where the compounds 1-6 are transparent. Fig. 4 shows the kinetic traces of singlet oxygen phosphorescence in the absence and presence of compounds 1-6.

Comparison of these kinetic traces shows major differences among compounds 1-6. The non-bridged control compound 1 did not suppress singlet oxygen generation. The lack of singlet oxygen suppression is consistent with the lack of observable quenching of singlet or triplet excited states of PpIX by 1 (Table 1). The bridged FRCC suppressed singlet oxygen generation to different degrees with 5 showing the largest suppression.

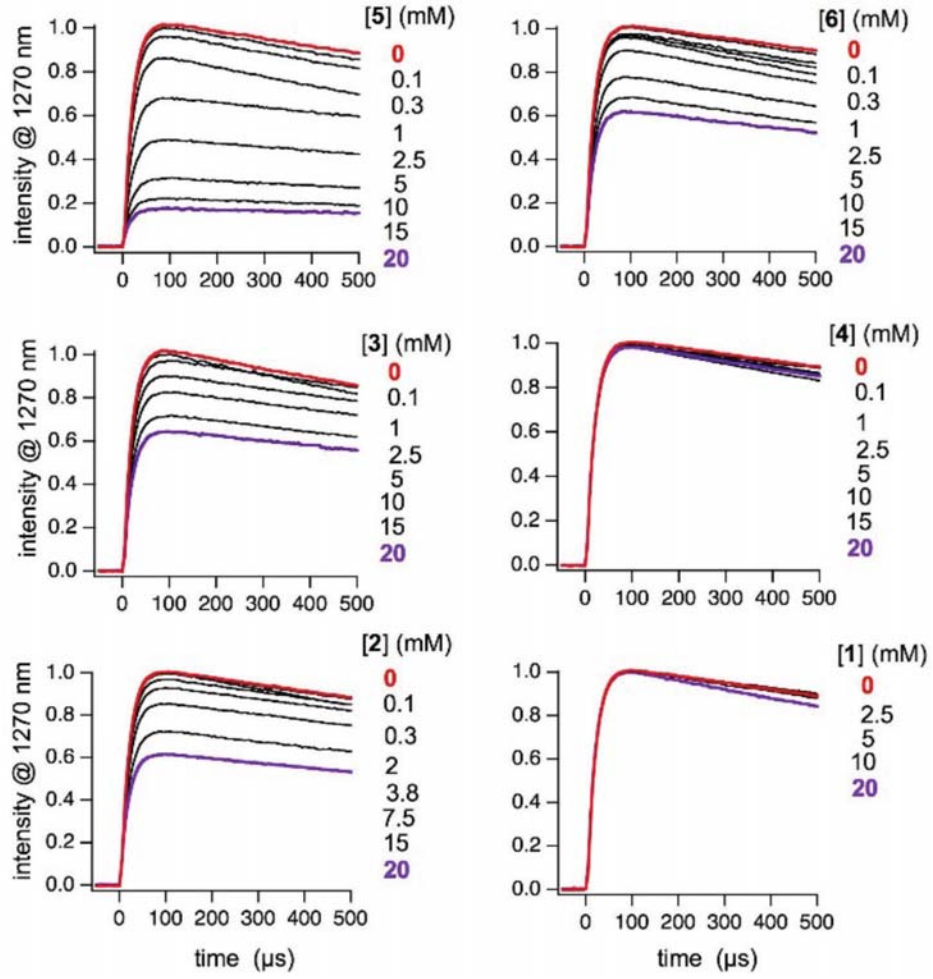

Fig. 4 Singlet oxygen phosphorescence traces monitored at 1270 nm generated by pulsed laser excitation at 532 nm of Pp-MeIX (17 µM) in air saturated CDCl3 solutions in the absence (red) and presence of variable amounts of compounds 1-6.

Stern-Volmer analysis of the data shown in Fig. 4 was performed to quantify the suppression of singlet oxygen generation by FRCC. The singlet oxygen phosphorescence intensity in the absence of stabilizer (I0) divided by the singlet oxygen phosphorescence intensity in the presence of stabilizer (I) was plotted against quencher FRCC concentration (Fig. 5). From the slope of these plots the Stern-Volmer constants were extracted and listed in Table 1. The Stern-Volmer constants are a direct measure of the singlet oxygen suppression efficiency of the stabilizers. Control compound 1 provides negligible or no singlet oxygen suppression. For FRCC 2, 3, and 6 Stern-Volmer constants of ~ 30 M-1 were observed. For these three stabilizers high PpIX singlet quenching rate constants (kqS ~ 5´ 109 M-1s-1) but low triplet quenching rate constants (kqT < 109 M-1s-1) were observed. For 2, 3, and 6, the singlet oxygen suppression is probably dominated by Pp-MeIX singlet excited state quenching. The highest Stern-Volmer constant was observed for FRCC 5 (240 M-1). Because of the very high PpIX triplet quenching rate constant (kqT = 3.2´ 109 M-1s-1), the high singlet oxygen suppression efficiency of 5 is probably dominated by triplet quenching.

In conclusion, FRCC 2, 3, 4, 5, and 6 are effective quenchers of PpIX's singlet excited state. PpIX singlet excited state quenching prevents the formation of PpIX triplet states which subsequently can generate toxic singlet oxygen. In addition, 5 is also an effective quencher of PpIX triplet states. With the help of FRCC, the formation of toxic singlet oxyen can be significantly suppressed,

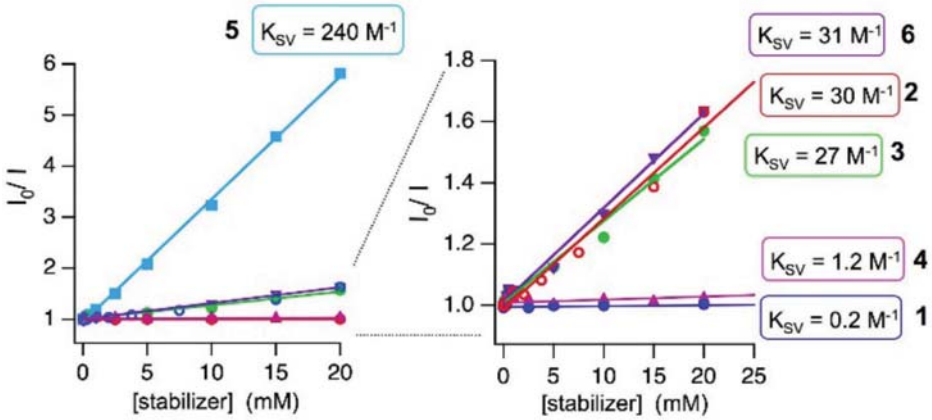

Fig. 5 Stern-Volmer plots of singlet oxygen phosphorescence data from Fig. 4.

indicating that photostabilizing endogenous porphyrins is an effective strategy for reducing cutaneous oxidative stress in human skin.

Experimental

Spectroscopy and measurements: Steady-state luminescence spectra were recorded on a Fluorolog-3 fluorometer (HORIBA Jobin Yvon). Fluorescence lifetimes were measured by time correlated single photon counting on an OB920 spectrometer (Edinburgh Analytical Instruments) in conjunction with a pulsed LED (PicoQuant) as excitation light source (496 nm). Singlet oxygen phosphorescence measurements were performed on a modified Fluorolog-3 spectrometer (HORIBA Jobin Yvon) in conjunction with a liquid nitrogen cooled Ge-diode detector (Model 403S, Applied Detector Corp.). A Spectra Physics GCR-150-30 Nd:YAG laser (532 nm, ca 5 mJ/pulse, 7 ns) was used for pulsed excitation to collect 1O2 phosphorescence decay traces at 1270 nm which were stored on a digital oscilloscope (TDS 360 from Tektronics). Laser flash photolysis experiments employed the pulses from a Nd:YAG laser (532 nm, 7 ns pulse width) and a computer-controlled system, as described previously.[15]

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The Measurable Fact about Skin Colour

by John Staton

Eurofins | Dermatest Sydney Australia

Abstract

For more than 100 years, science has used various measures to classify humans into skin types, based on colour. History of these classifications is discussed – how they have been used and relied upon for not only placing humans in colour boxes, but also for decision making in clinical studies.

An argument is presented for abandoning the widely used Fitzpatrick Scale for cosmetic science purposes, using the example of changes now imminent for the SPF testing of sunscreens according to the Reviewed ISO 24444 test method, most likely to be adopted into Australian New Zealand Standard AS/NZS 2604 in 2019-20.

History of Skin Colour Evolution – West meets East

It is estimated that modern humans evolved around 150,000 years ago in Africa and migration to other geographic locations began around 100,000 years ago (1).

Dark, heavily UV protected skin was the original skin colour but, as part of the ongoing evolutionary process, and most likely influenced by Neanderthal man from more northern climates, differing skin colour resulted, apparently influenced by geographic relocation to latitudes further from the higher UV light areas (2).

Around late 18th century, attempts to classify geographic populations by race began to appear in the literature. An early example is Nordisk Familjebok, a Swedish encyclopedia dating from 1904 (3).

First Objective Measurement

Whilst dress and other cultural specificities prevailed at this time, skin colour became the interest of researches in racial diversity. Such was the interest of Felix von Luschan, then Ethnologist at Museum of Berlin. In 1897, von Luschan developed an objective chromatic typing scale based on coloured plates graded into 36 tones (4) Fig 1.

This, he and others used to define skin type. Von Luschan's scale was in use at least as late as the 1940s as shown in Fig 2.

Subjective Replaces Objective

In 1975, Thomas B. Fitzpatrick (5) proposed, then a researcher in the

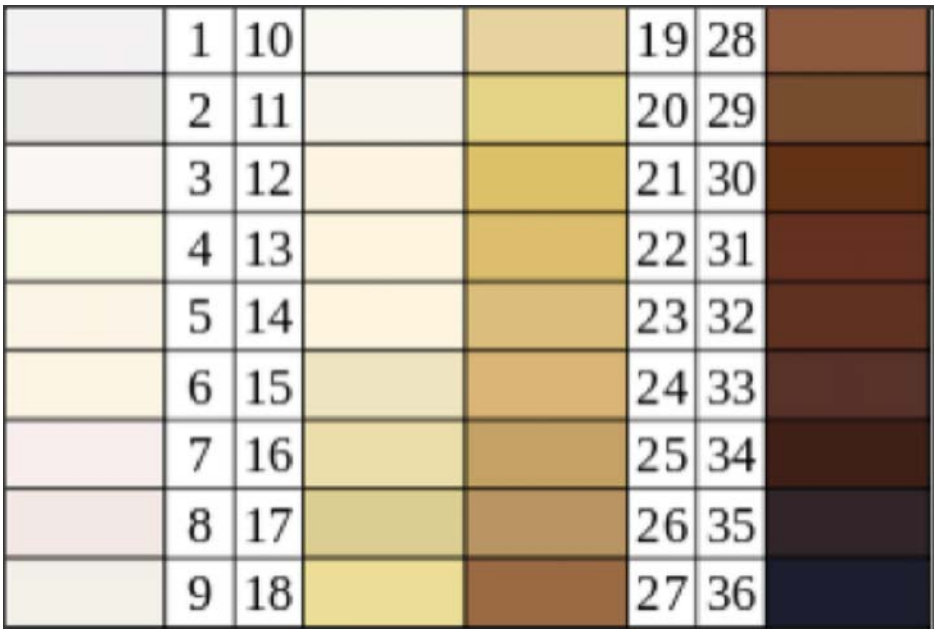
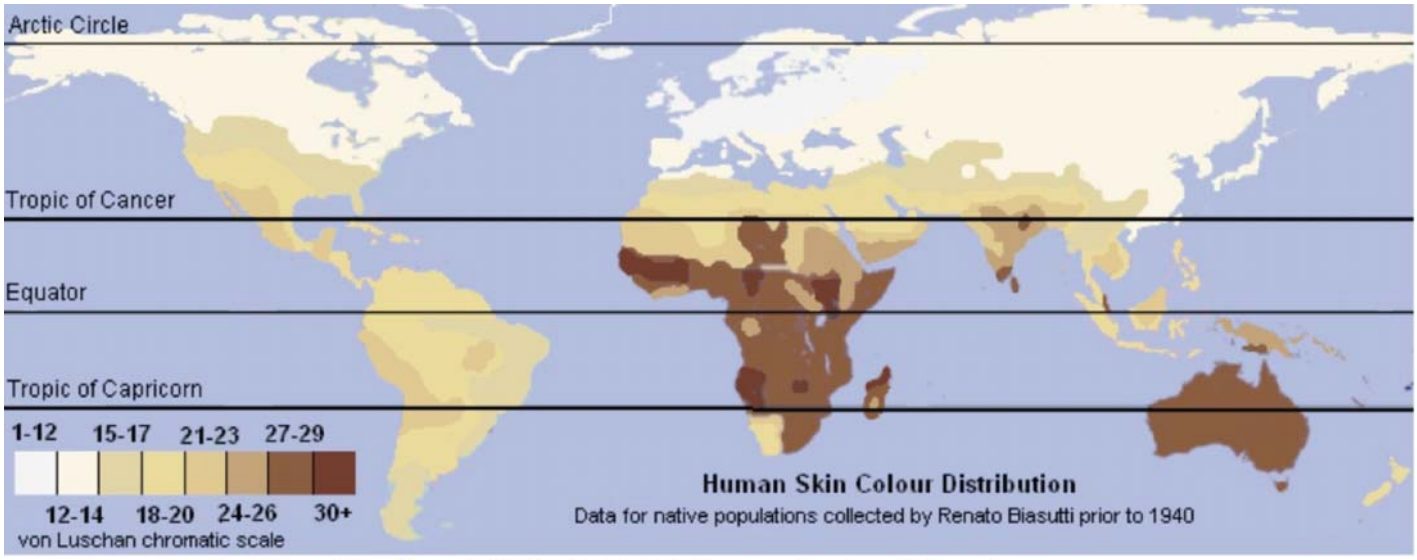


Fig 1. Feiix von Luschan Chromatic Scale



http://en.wikipedia.org/wiki/File:Unlabeled_Renatto_Luschan_Skin_color_map.png

Fig 2 – a geographic skin colour mapping.

Department of Dermatology at Harvard Medical School and discoverer of human tyrosinase and the metabolic pathway for melanin, the melanosome and basic melanin synthesis, used a subjective classification system to explain the differing skin responses to tanning . This was the Fitzpatrick Skin Typing and set out descriptors to classify individuals into six skin types. Although mostly subjective, this system became widely used in dermatology and entered the methodology for Sun Protection Factor testing in early versions of standards, including that introduced in Australia in 1983 (6) ARPANSA (7) and Cancer authorities (8) still rely on these classification for educational health care advise related to sun safety.

Colour Typing for Sunscreen Testing

Traditionally, for the purposes of classification of test volunteers for enrolment into SPF testing, Fitzpatrick Types I, II and III are recruited and classified (9) (10).

Skin type I—skin which burns and never tans.

(Skin type II—skin which burns readily and tans slightly.

Skin type III—skin which burns and tans moderately.

Skin types above III are not recruited due to difficulty in observing the erythemal dose endpoint of the test.

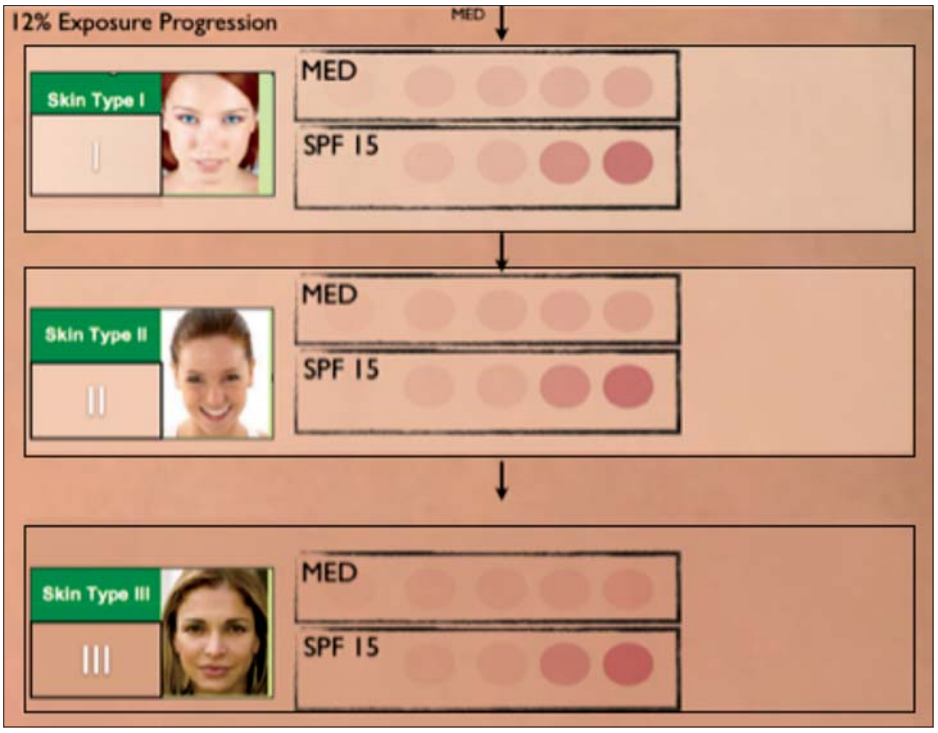


Fig 3 – Skin Types I,II and III Erythemal end point progression

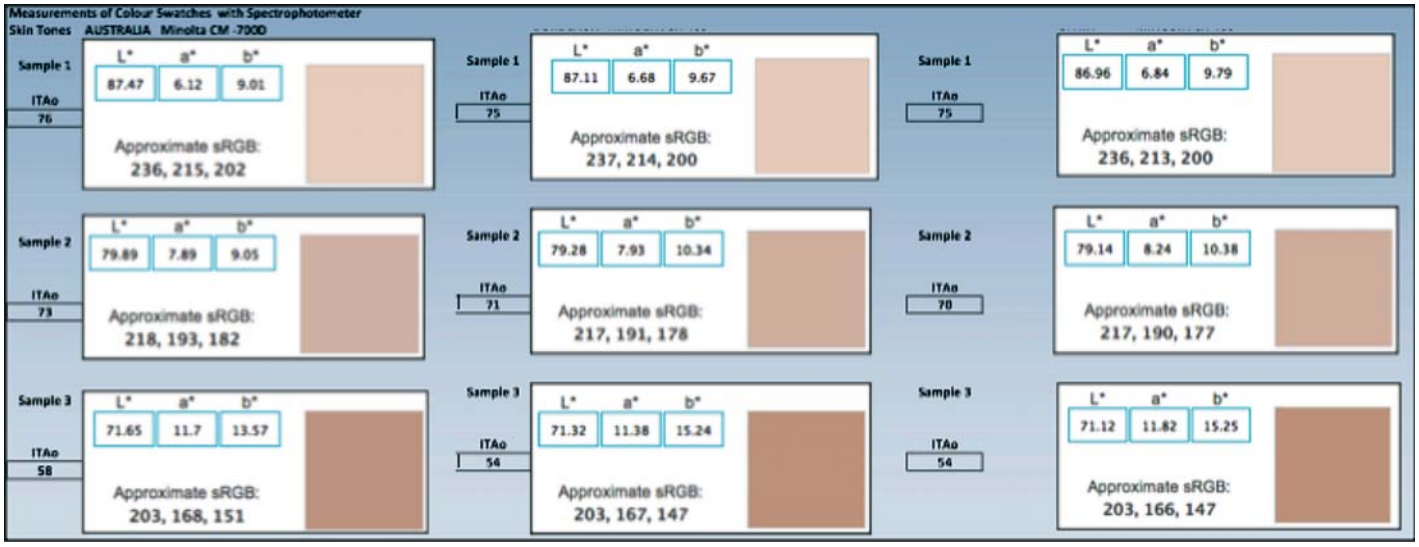
Fig 3 illustrates this – as background colour changes, end point becomes less observable.

As opposed to SPF testing, darker skin types can be used for UVA protection testing, where the endpoint is tanning and not erythema.

Whilst Fitzpatrick has been shown to be useful, it is far from the ideal tool for the classification of test subjects for sun product testing. Marketers of colour masking cosmetics well know the need to provide more than three pigmented shades to cover skin colour, which in reality, is a continuum.

Instrumental Colour Classification

The potential to measure colour digitally by colour computer became available in 1940 following the invention by Richard Hunter of the tristimulus color model and was described by Chardon et al in 1991. (11). With the use of a colour space known as L*a*b* (12), we are able to precisely quantify the colour of skin rapidly and reliably. This technique, known as Individual Tangent Angle "ITAo", was introduced as an option into the first edition of ISO 24444 (10) and then ISO 24442



Australia *France* *Spain*

Fig 6 – Colour Calibrations between Test Labs

(13) test methods although Fitzpatrick classification was still retained in these first edition standards.

The ITAo measures the "L*" values which is the black to white, or grey scale component whilst the "b*" value is the yellow-blue. This conveniently leaves the "a* " component – blue to red out of the classification – see Fig 4.

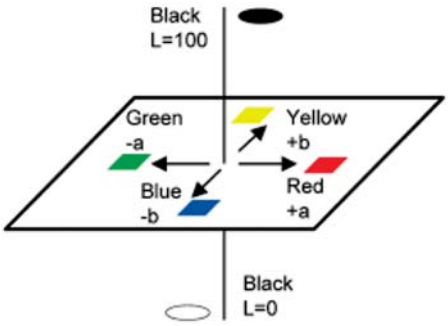


Fig 4 – Colour Space showing L*, a* and b* components

The ITAo is scored from the equation shown in Fig 5.

$$ITA = \left\{ \text{arc tangent} \left[\frac{(L^* - 50)}{b^*} \right] \right\} \frac{180}{3{,}141\,59}$$

Fig 5. ITAo Calculation

The relevance of this is that the "red" is the erythemal endpoint which is, at this time, visibly accessible. Red is also the color of flushed skin and those familiar with evaluating test subjects newly arrived, late and hot and bothered, will be aware that this component is not part of normal skin colour. Essentially, ITAo does not change over life and is thus the truest quantification of our individual skin colour.

The reliability of the measurements of ITAo can be evidenced. Using paint colour swatches close to the three Fitzpatrick Skin types, using Minolta hand held spectrophotometers, we measured instruments in different labs and the results can be seen in Fig 6.

Conversely, a review of our historical data on n= 331 test subjects where subjective classification of Fitzpatrick was compared with objective ITAo, showed 13% Mismatches. The implication of this is that test subject exposure calculations cannot be made reliably when running SPF tests. Fig 7 shows how it is not possible to correlate Fitzpatrick when assessing UV light response versus skin type, whereas ITAo is entirely predictable.

Further evidence of predictability of ITAo is shown when results from "Asian" skin are isolated in Fig 8. This evidence, from our own measurements, indicates that ITAo allows the mix of skin types to be unnecessary when conducting SPF testing. Under Fitzpatrick, it was believed that examples from all three types was necessary.

In effect, what this is indicating is that the SPF number we certify in the test IS

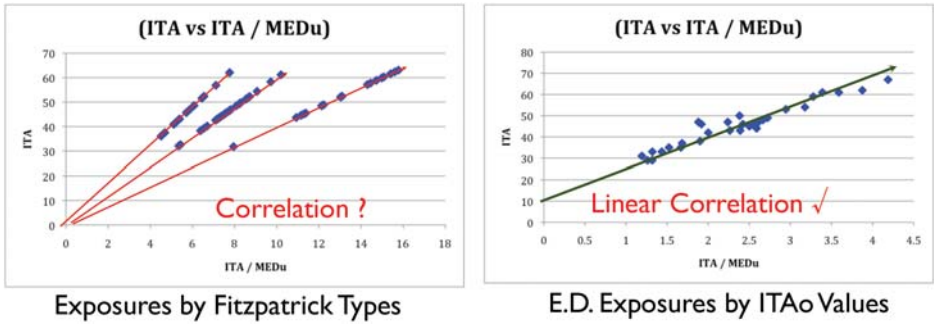


Fig 7 – Fitzpatrick driven versus ITAo driven Correlation

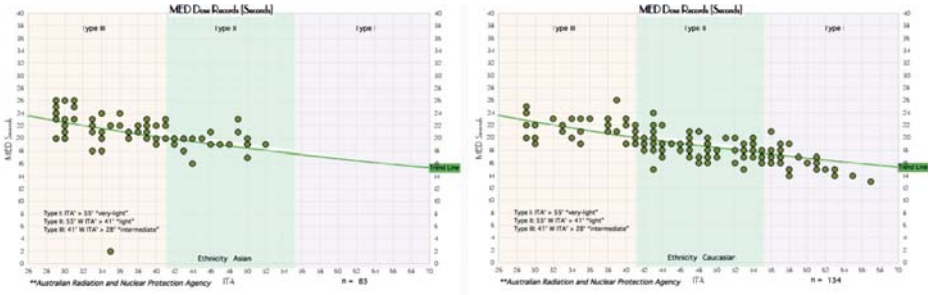


Fig 8 – Asian skin MED/ ITAo (left) versus Caucasian Skin

relevant for all skin types and geographic zones.

Review of ISO SPF and UVA in vivo Test Standards

Based on data presented by this Author and subsequently supported by other complementary data, Working group 7 of ISO 217 Technical Committee has recommended that Fitzpatrick classification be deleted from both ISO 24444 and ISO 24442 Standards in their current reviews. From 2019 onwards, only ITAo may be used for skin classification. A guidance of erythemal response expectations for a Minimal Erythemal Dose "MED" versus ITAo will be included – see Fig 9.

Conclusion – The Beauty of Opportunity

Whilst we see the end of Fitzpatrick as a tool for SPF testing, we have not yet utilized the opportunity to use the ITAo for education of our customers in a truly targeted and personalised way. Apart from the recognition in colour cosmetic ranges, we defer to Fitzpatrick and mostly ignore the original system of von Luschan who no doubt would have delighted in using the spectrophotometer in his research into measuring the skin colour for his fact finding.

On the positive side, the reporting of ITAo at least for SPF improves our confidence in the reliability and repeatability of this test methodology.

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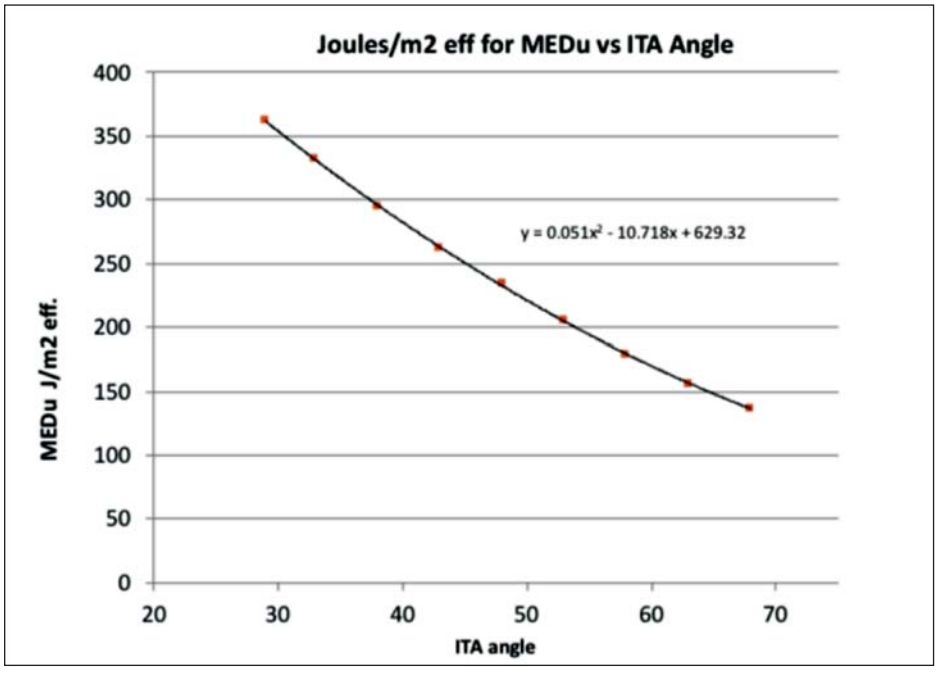

Fig 9 – Dose Guidance for MED response from DRAFT ISO 24444 (2019)

Buddleja officinalis, Flower Extract –

BOFE – an innovative active with global photoprotection properties

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Abstract

External atmosphere is wild and aggressive environment: Sun generates various radiations (ultraviolet, visible light, infrared: IR) and excessive exposure of skin leads to free radicals formation, inflammation, apoptosis and extracellular matrix degradation. These are main causes of premature aging and various disorders, including short-term (sunburns, dryness, erythema . . .) and long-term consequences (dark spots, photoaging and cancers). Protection against UV alone is not sufficient to prevent skin disorders: approximately 50% of free radicals in skin originate from visible light and IR. Harmful effects of blue light from artificial sources (smartphones, screens . . . more concentrated in blue than natural light) become important issue for public health and skin aging. From *Buddleja officinalis,* adapted to high levels of sunrays exposure in Chinese mountains by exceptional richness in phenylpropanoids, we developed an innovative global photoprotector: "BO Flower Extract – BOFE" – concentrated in verbascoside and echinacoside – protects against damaging effects of UV, blue light and IR."BOFE", by powerful antioxidant properties, preserves skin barrier integrity and maintains homeostasis. Maintaining normal skin physiology by counteracting responses to different radiation-induced stresses, "BOFE" prevents dark spots, premature wrinkling and preserves skin quality and radiance.

Introduction

Intrinsic and extrinsic aging are two processes of skin aging. Extrinsic aging results from external factors, such as smoking, exposure to ultraviolet radiation (UVR) often known as photoaging, and atmospheric pollutants. While reactive oxygen species (ROS) are continuously produced in the skin by fibroblasts and keratinocytes and are involved in physiological processes. ultraviolet A (UVA) and to a lesser extent UVB are able to increase the levels of singlet oxygen (1O2), superoxide anion (O2.-), hydroxyl radical (.OH), and hydrogen peroxide (H202). In addition to direct DNA alterations, which include DNA base damage, DNA single- and double-strand breaks, and cross-linking of DNA and proteins, UVA/B-generated ROS modulate a number of signal transduction pathways (e.g., MAPKs) and transcription factors (e.g., NF-κB) that are important in regulating genes involved in the pathogenesis of inflammation (interleukins, cyclooxygenase[2] -COX-2) and in the control of the cell cycle and apoptosis (e.g., cyclin D1, Bcl2, and p53). Activation of transcription factors via ROS is achieved by signal transduction cascades that transmit the information from outside to the inside cell. Tyrosine kinase receptors, most of the growth factor receptors, such as epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGF) and serine/ threonine kinases are target of ROS. ROS and EGFR activation play central roles in UV-induced matrix metalloproteinases (MMPs) expression through initiating extracellular signal-regulated kinase (ERK)–mediated AP-1 signaling. The specific role of MMPs in photoaging has been suggested by the observation that UV irradiation of human fibroblasts and the skin induces the expression of collagenase (MMP-1), stromelysin-1 (MMP-3) and 92-kDa gelatinase (MMP-9). Activation of MMPs is responsible for the degradation of skin collagen and inhibiting collagen synthesis of extracellular cellular matrix. ROS and degrading enzymes, which will subsequently lead to damage in

surrounding tissues, are the result of the inflammation of the skin, which is associated with the influx of immune cells. Skin inflammation attracts immune cells with the subsequent rise of oxidative stress that may promote skin aging.

Cytoprotective responses are characterized by the upregulation of antioxidant enzymes and decreased sensitivity to oxidative stress damage[3] (Finaud et al., 2006). Various compounds with different antioxidant properties are found in plants, which may be applicable as therapeutics to decrease and prevent free radical damage. In this way, several medical plants have been screened and assessed for properties in antagonizing free radical-induced oxidative stress. However, protection against UVR alone is not sufficient to prevent deleterious dermal consequences. Over 50 % of these free radicals are generated in the visible and infrared spectral regions[13] (Widel et al., 2014). In fact, the exposure to infra-rouge (IR) radiation induces biological effects, which are similar to UV action, including activation of MMP-1 and decrease of the collagen synthesis and its degradation. Concerning the molecular mechanisms involved, it was shown that high doses of IR radiation (>120 J.cm-2) induce activation of mitochondrial cytochrome C oxidase and electron transport chain with the formation of ROS such as H2O2, 1O2,[10] (Schroeder et al., 2007). IR exposure also induces an increase in the skin temperature that can be a consequence of the generation of heat shock radicals [1](Akhalaya et al., 2014). Therefore, IR exposure promotes premature skin aging by degradation of the extracellular matrix.

Besides, artificial sources of blue light (390-500nm) or high-energy visible light (HEV) such as light-emitting diodes (LEDs) are also becoming an important issue. Due to its high energy, HEV has a greater dispersion than others wavelengths from visible spectrum. Damages from HEV radiations in retina have been widely documented in last 30 years with several studies in mouse, primates and in vitro (Kuse et al., 2014). Blue light induces an increase in oxidative stress, mainly at wavelengths below than 430 nm.

Thus, generally speaking, skin is a major target for oxidative stresses and damage [12] (Trouba et al., 2002) since it is continually exposed to ROS, which are involved in basic cellular processes such as signal transduction, gene expression, and apoptosis and possess both detrimental and beneficial roles. Skin ROS are involved in redox homeostatic maintenance and thus may be involved in the development of various skin diseases including inflammatory processes and cancer [14] (Wölfle et al., 2014). A possible approach to attack ROS-mediated disorders for both preventive and treatment means, is based on the use of photoprotective substances, which can be found in plants as secondary metabolites. Among them, phenylpropanoids (PPS) substances have the ability to absorb UV. Numerous studies have been focusing on the molecular mechanisms of biological activity of natural PPS. These mechanisms include the suppression of both the production of IL-1B and its effects on the activation of NF-κB, the activation of caspase [3], inhibition of the transcriptional activity of the COX-2 gene [5] (Korkina et al., 2007). It is interesting to note that PP-mediated inhibition of NF-kB activity occurs without degradation of the cytoplasmic NF-kB inhibitory protein I kappaB alpha, suggesting that the molecular target for PPs is at post –IkB degradation level. All these results provide attracted to natural PPs from cosmetic product manufacturers. *Buddleja officinalis (BO)*, a shrub in the Buddlejaceae family is known in traditional medicine for their wound healing, anti-inflammatory, diuretic, anti-allergic detergent, antiviral and antibacterial properties. The principal components of the *Buddleia officinalis* extracts were identified as phenylpropanoid derivatives such as verbascoside, and echinacoside [11] (Thai et al., 2009). Verbascoside, also known as acteoside, has several biological activities including neuroprotective, anti-tumor, antibacterial, photoprotective, and antioxidant (Seo et al., 2013; Alipiera et al., 2014). Furthermore the anti-inflammatory activity of verbascoside has been noted by an *in vitro* test performed on cell cultures of primary human keratinocytes [5] (Korkina et al., 2007), in which verbascoside was able to significantly reduce, in a dose-dependent manner, the release of pro-inflammatory chemokines. An *in vivo* study, conducted on inflammation of the intestinal mucosa, demonstrated that verbascoside is able to inhibit the activation of pro-inflammatory proteins and consequently the enzymatic activity of matrix metalloproteinases, the latter also being involved in skin aging phenomena [8] (Mazzon et al., 2009).

Based on these interesting data, the aim of this present study was to evaluate the photoprotective effect of active PPs extracts from BO (BOFE) using *ex viv*o and *in vitro* tests. Firstly, we examined the high-energy visible (HEV) light protection capacity of BOFE on human Keratinocytes in vitro. Secondly, we evaluated the effect of BOFE on infrared-induced MMP-1 release on normal human dermal fibroblasts. Finally, the effect of BOFE on UV-induced lipid peroxidation with a non-invasive *ex vivo* method using tape strips of outermost layers of *stratum corneum (SC)* from human volunteers was examined.

Experimental section

1) *Effects of BOFE in counteracting oxidative stress induced by HEV*

Human keratinocyte cells (HaCat line) were cultured overnight at a 10.000 cells/well of density in a 96 well plate, in growth medium. 24 hours later, the culture medium was removed and substituted for new culture medium supplied with the BOFE at different concentrations (0.01, 0.03 and 0.1%) during 24 hours. After treatment, cells were irradiated in the cell culture plate during 20 minutes. Light source was at 6 cm from cell surface resulting in an approximate dose of 7 mW.cm-2, at a wavelength of 420nm. Immediately

after irradiation, the ROS detection buffer was supplied into the cell culture medium and incubated during approximately 1 hour. The intracellular ROS accumulated reacted with a fluorogenicprobe localized in the cytoplasm, resulting in a fluorometric product in amounts proportional to the amount of ROS present. Fluorescence quantification is measured at λex=490/ λem=525. The mean background of fluorescence was subtracted to each measure in each replicate. Then, the mean values for the four replicates in the control (untreated, non-irradiated) and the control + HEV (untreated, irradiated) were obtained and these values were used to normalize each measure of fluorescence of the replicates in the corresponding samples and conditions. By last, to determine the HEV-induced damage protection of the different treatments, ROS basal levels of the control were subtracted from the ROS levels of the control + HEV, and this value was used to normalized each measure of luminescence of the replicates in the corresponding samples and conditions.

2) *Effect of BOFE on infrared-induced MMP-1 release on normal human dermal fibroblasts.*

Normal Human Dermal Fibroblasts (NHDF) were cultivated in a 24 well plate, in growth medium. 24 hours later, the culture medium was removed and substituted by new culture medium supplied with dexamethasone (10-7M) or BOFE at 0.1% during 24 hours. After pre-treatment, the growth medium was replaced by the irradiation medium and cells were irradiated with infrared (0.64 kJ.cm-2) during 1 hour. After irradiation, irradiation medium were removed and substituted by new growth medium supplied with dexamethasone (10-7M) or BOFE at 0.1% during 48 hours. Two experimental replicates (treated and controls) were evaluated for MMP-1 release.

MMP-1 release was evaluated in the culture medium supernatants by ELISA kit according to manufacturer's instructions. The percentage of protection from infrared- induced MMP-1 release was calculated according to:

Protection (%) = ((irradiated control Mean – irradiated sample Mean)) / ((irradiated control Mean) – (non-irradiated control Mean)) * 100

3) *Determination of the UV-protective effect of BOFE by the ex vivo evaluation of the lipid peroxidation (LPO)*

All participants gave written informed consent before entering the study. 15 healthy Caucasian women with photo types II and IV were included. Formulation containing 2% of BOFE was applied by each volunteer twice a day over a period of 14 gays on forearm. A placebo emulsion without BOFE was applied on the other forearm as control. The volunteers refrained from using cosmetics, body oils, sunscreens or moisturizers on their arms 7 days prior to the study, and during the study except the two formulations selected.

The tape stripping of the SC of each forearm was carried out on the 14th day using D- squameTM tapes (CuDerm, Dallas, USA) (4 trips per each treated area per volunteer). Then, half of the strips were fixed in a glass plate and irradiated during 1h using a light source simulating UV solar (UVA = 28mJ.s-1.cm-2). Next, the SC lipids were extracted from the strips with methanol and lipid peroxidation was measured with thiobarbituric acid reactive substances assay (TBARS measurement) by spectrophotometry at 534nm, as previously described [2] (Alonso et al., 2009).

Figure 1 presents the schedule of the protocol. The quantity of LPO formed from the placebo-treated skin, non-irradiated (Pni) and irradiated (Pi), and the amount of LPO formed on BOFE-treated skin, non-irradiated (Sni), respectively, were calculated from absorbance values obtained of SC extraction samples by malondialdehyde (MDA) determinations. The percentage of LPO inhibition (LPO% inhibition) was determined from the difference in values of amount of LPO formed between the SC placebos and SC BOFE samples, applying the following calculations. LPO (%inhibition) = (1-(Si-Sni)/(Pi-Pni)).100

Statistical analysis

All the data were normalized to the control and represented as mean ± standard deviation, Tests applied for the analysis were the Student's t-test in the three experimentations. Statistical significance was set at $p<0.05$, 95% of confidence.

Results

1) *Effects of BOFE in counteracting oxidative stress induced by HEV Figure 2 represents ROS accumulation in samples treated with BOFE. HEV light during 20 minutes*

HEV light during 20 minutes induced ROS levels in human keratinocytes in vitro by 23.5 ± 0.8 %, compared to the control group. When human keratinocytes were treated with the products at each concentration and irradiated, results showed that BOFE at 0.01 %, 0.03 % and 0.1 %, significantly decreased ROS levels, compared to the Control + HEV group as shown in Figure 2. Results showed that the treatments with BOFE at 0.01 %, 0.03 % and 0.1 % significantly protected from HEV-induced oxidative stress by 37.0 ± 5.2 % ($p < 0.001$), 41.9 ± 4.1 % ($p < 0.0001$) and 42.9 ± 4.7 % ($p < 0.0001$), respectively.

2) *Effect of BOFE on infrared-induced MMP-1 release on normal human dermal fibroblasts.*

In non-irradiated conditions, MMP-1 basal production by NHDF was 37 ng.mL-1. Infrared irradiation (0.64 kJ.cm-2) induced an increase in MMP-1 release by 765% compared to the control group (283 ng.mL-1). Dexamethasone at 10-7M drastically inhibited this increase (18 pg.mL-1) leading to a complete protection from infrared irradiation. BOFE at 0.1% significantly inhibited the IR-induced MMP-1 release by 22% (220 pg.mL-1).

3) *Determination of the UV-protective effect of BOFE by the ex vivo evaluation of the LPO*

Lipid isolated from SC of BOFE-treated area presented a statistically significant inhibition of UV-induced peroxidation by 20% compared to placebo treated area ($p < 0.001$). 67% of the volunteers demonstrated an inhibition of UV-induced lipid peroxidation in BOFE-treated area (Figure 4).

Discussion

The skin is a major target of oxidative stress which can be developed into oxidative damage if not blocked. In the dermo cosmetic field, the topical application of antioxidants is often suggested as a possible strategy to prevent and modulate oxidative skin damage. In recent years, there has been considerable interest in using botanical agents to prevent skin damage resulting from solar UV-irradiation. In this context, we investigated the photoprotective effect of active phenylpropanoids extracts from BO (BOFE) using ex vivo and in vitro tests. Three major findings emerged from our study:

1) When analysing the HEV-induced damage (difference between ROS levels in the « Control + HEV » and the « Control »), results showed that the treatments with BOFE at 0.01 %, 0.03 % and 0.1 % significantly protected from HEV-induced oxidative stress.

2) BOFE at 0.1 % significantly protects from IR-induced MMP-1 release by 22% compared to irradiated condition. Thus, it appears that BOFE preserves extracellular matrix and prevents its premature degradation induced by infrared.

3) Lipid isolated from SC of BOFE treated area presented an inhibition of UV-induced peroxidation, compared to placebo treated area. Infrared (IR) radiations penetrate deeply into the skin and approximately half of the IR is absorbed in the dermis. Even though the negative effect of solar radiation on human skin is usually associated with exposure to UVB and UVA radiation, many studies show that near-infrared (NIR) and especially IR radiations at high doses can negatively affect the human skin [1] (Akhalaya, Maksimov et al. 2014). The exposure to IR radiation, therefore, induces biological effects, which are similar to UV action, including activation of MMP-1 and decrease of the collagen synthesis and its degradation. Our results showed that PPS extract from BO, in particular verbascoside, and echinacoside, have the ability to reduce the degradation of extracellular matrix degradation and photoaging. These results are in line with those of Hwang et al., (2011), who found that verbascoside reduced the level of MMPs expression through the suppression of NF-κB activation.

[9] (Opländer et al. 2013) reported that irradiation with blue light led to intracellular oxidative stress and toxic effects in a dose and wavelength dependent manner. This toxic effect on the skin was to be related to the generation of non-enzymatic nitric oxide (NO). Furthermore, blue light at low doses reduced the antioxidative capacity of fibroblasts. Finally, some studies showed that the irradiation of the human skin with blue-violet light results in a depletion of the epidermal antioxidants associated with a degradation of cutaneous carotenoid and long-lasting hyperpigmentation [9] (Opländer et al. (2011). Our results revealed that in vivo, BOFE significantly reduces the lipoperoxydation products induced by blue light (Figure 2). We also noted in volunteers that BOFE protects against UV-induced lipid peroxidation. It has yet been shown that verbascoside promotes skin repair and ameliorates skin inflammation due to its ROS scavenging, antioxidant, iron chelating, and glutathione transferase (GST) activity inducing properties [7] (Kostyuka et al., 2011). Vertuani et al., (2011) also revealed that verbascoside increased antioxidant activity.

Conclusion

UV exposure causes skin damage, and chronic exposure carries a risk of skin cancer. New strategies are needed to combat UV skin damage. Our results showed that topical administration of BOFE protects against UVB-induced skin damage in women. Moreover, using in vivo protocol, BOFE reduces the production of ROS induced by blue light and the level of infrared-induced MMP-1 release. The present findings demonstrate that BOFE is endowed with good in vivo skin photoprotective properties and that this is likely due to the PPS content of BO (verbascoside and echinacoside). Therefore, these results suggest that BOFE may reduce the degradation of the protein matrix and the lipid peroxidation, thus preserving the integrity of the cutaneous structure, thanks, particularly, to the protection of the membranes, which are essential for cellular activity. Thus, BOFE may exhibit some potential to prevent photo-damage. Additional studies to better understand the mechanism of action of BOFE responsible for its photoprotective effects are in progress in our laboratory.

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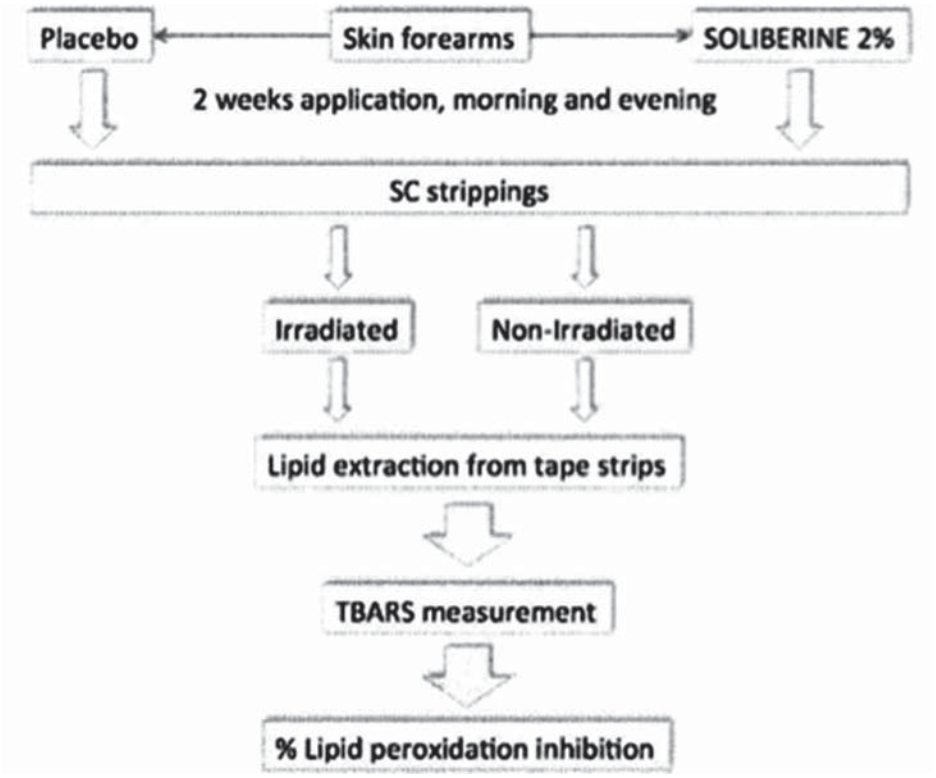
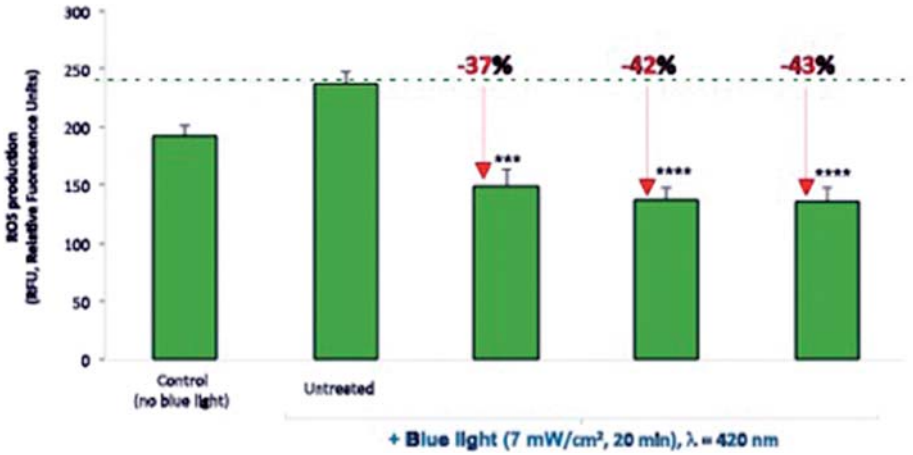


Figure 1: Protocol used for analysis of UV-induced lipid peroxidation (LPO) in Stratum corneum of human volunteers.



BOFE 0.01% BOFE 0.08% BOFE 0.1%

Figure 2: Bar graphs represent the HEV-induced ROS accumulation in samples treated with BOFE (0.01 %, 0.03 % and 0.1 %). Control vs treated: *** p< 0.001; **** p < 0.0001.

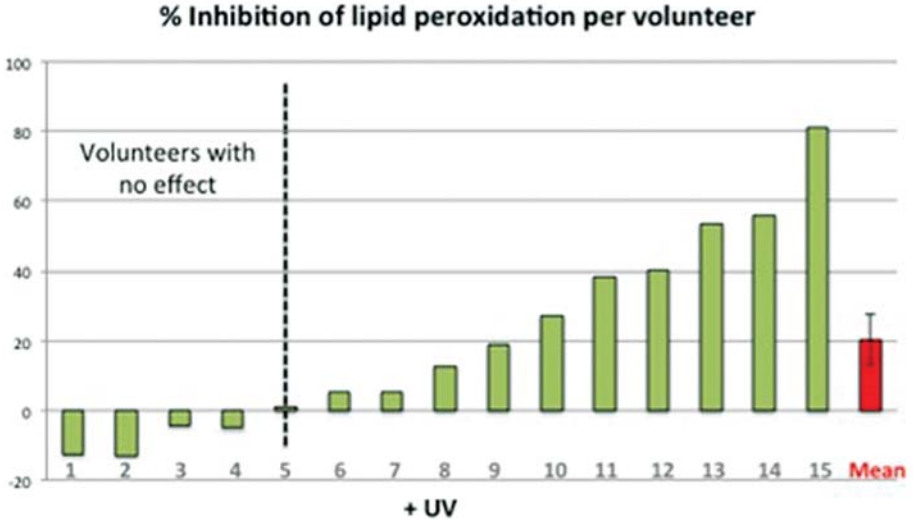


Figure 4: Lipid isolated from Stratum corneum of BOFE 2%-treated area for each volunteer

Biomemetic and Psychobiological Approaches for a Positive Skin Aging – Effect on Senescence Skin Markers

by Edwige Ranouille[1], Marion Napoli[1], Sandie Gervason[1], Jean-Yves Berthon[1], Edith Filaire[1,2]

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Abstract

Senescence is involved in the development of age-related diseases and the loss of tissue functionality with age. Even if senescent cells can have beneficial functions in wound healing, accumulation in relation with aging alters the surrounding tissue. In fact, senescent cells have a secretion pattern called Senescence-Associated Secretory Phenotype that comprises a complex mix of factors including cytokines, growth factors, and matrix metalloproteinases. The cosmetics industry has focused on bioactive substances derived from natural products such as plants and marine algae. Besides these products, bacteria seem to prevent immune-senescence. During the past 10 years, a lot of R&D works have been done to isolate an active ingredient from bacteria strains. *Sphingomonas* strains, which have been isolated from a variety of environments, have the ability to utilize a wide range of organic compounds and to grow and survive under low-nutrient conditions. In order to get more information about the potential benefits of bacteria extract over skin aging, we evaluated the effect of *Sphingomonas* sp (SP) on an aged full-thickness skin equivalent model. We showed that it attenuates cellular senescence thanks to important markers: inhibition of p21 and p16 transcription factors but also increases the expression of fibrillin-1 and versican. Moreover, it decreases IL-6 and IL-8 concentrations. *In vivo* study in 24 women aged from 60 to 70 years shows that the bacteria extract maintains skin's beauty after 60 years old by maintaining the cutaneous barrier and by improving the suppleness of the skin. It has the capability to restructure and has and an anti-aging effect. Moreover, application of SP during 56 days has significant benefits on psychological health. Therefore, SP can be used as an anti-aging cosmeceutical agent, suggesting unique properties of proteobacteria for future investigations on topical applications.

Key words: senescence, bacteria, positive aging

Introduction

Aging is the result of a gradual functional decline at the cellular, and ultimately, organismal level, resulting in the development of myriad chronic illnesses including heart disease, stroke and diabetes. On a cellular level, aging was first described by Hayflick and Moorhead (1961), who demonstrated that somatic mammalian cells have a finite propensity for cell division, after which they enter an irreversible growth arrest termed cellular senescence. Today, senescent cells are characterized by their inability to proliferate, to resist to apoptosis, and to secrete factors that promote inflammation and tissue deterioration (He and Sharpless, 2017). It is now clear that several types of cellular stressors can trigger senescence. These include telomere shortening and dysfunction (Takai et al., 2003), inadvertent activation of oncogenes (Suram et al., 2012), changes in chromatin structure and epigenetic stress (Munro et al., 2004), oxidative stress, mitochondrial dysfunction (Wiley et al., 2016), or persistent activation of DNA damage checkpoints (Rodier et al., 2009).

All these stimuli induce cell senescence through two main pathways 1) a p53-p21 (also called p21Cip1) signaling pathway that is partially telomere dependent 2) a p16 (also known as p16INK4a) – pRb (retinoblastoma tumor suppressor protein) pathway that is independent of telomere dysfunction. These pathways

are dissociated with one another and can individually induce senescence, although there is also overlap and interaction (Van Deursen, 2014). The suppression of cyclin-dependent kinases (CDKs) is produced by both these transcriptional activation. When activated, p53 inhibits cell proliferation via activation of its transcriptional target p21. Both p21 and p16 maintain the protein pRb in its hypophosphorylated and active state. Active pRb suppresses the E2F1 (a member of E2F family of transcription factors, which induce gene transcriptions that are essential for cell proliferation)-dependent expression of genes that regulate progression of the G1/S phase of the cell cycle, and thereby irreversibly blocks cell cycle entry (Gire et al., 2015) (Figure 1).

Removal of senescent cells accumulated in the body during aging alleviates atherosclerosis, tumor development, and functional declines of heart, kidney, and fat tissues, resulting in prolonged healthspan and lifespan (Baar et al., 2017). These effects may be attributable to so-called senescence-associated secretory phenotype (SASP), whereby cells secrete high levels of inflammatory cytokines, chemokines, growth factors, and metalloproteinases (MMPs). The most prominent cytokine of the SASP is interleukin-6 (IL-6), a pro-inflammatory cytokine. IL-6 has been shown to be associated with DNA damage and oncogenic stress-induced senescence of mouse and human keratinocytes, melanocytes, monocytes, fibroblasts, and epithelial cells (Coppe et al., 2008). Generally speaking, the SASP in senescent cells can induce senescence in neighboring cells, alters the behavior of surrounding cells and tissue homeostasis by activating various cell-surface receptors and their signal transduction pathways, and induces tumorigenesis and malignant progression of nearby premalignant cells. Secretion of several pro-inflammatory and tissue-remodelling factors by senescent cells might contribute to the loss of tissue homeostasis and unbalanced tissue structure. While short-lived senescent cells may act as positive regulators of wound healing, the presence of long-lived senescent cells may exacerbate pathological diseases in the skin. Indeed, the chronic secretion of MMPs by senescent cells might be an important contributor to the degradation of collagen and other extracellular matrix components in the dermal connective tissue, a hallmark of skin aging (Fisher et al., 2013). A persistently elevated number of senescent cells may disrupt cell-signaling responses and prevent wound repair to progress through the different stages of the healing process, one of the main features of chronic wounds.

Expression of β-galactosidase (SA-β-gal) is known to be one of the well-characterized and simplified methods to detect senescence *in vitro* culture cells as well as for aged tissues *in vivo* (Dimri et al., 1995). The expression of inflammatory cytokines (IL-6) or chemokines (IL-8) has been extensively used as biomarkers for measuring senescence in cells and in tissue. Increased levels and/or activity of p16, p53 and p21 have been shown to be associated with cell senescence and are also considered as important biomarkers of cell senescence and tissue aging.

The accumulation of senescent cells with age is thought to contribute to impaired tissue homeostasis and to different age-related diseases. Epithelial tissues in humans are constantly renewing and the skin represents the gold standard example of an epithelial tissue continuously regenerating. Since proliferation of stem and differentiated cells is a major contributor to skin renewal, the accumulation of an excessive number of senescent cells may cause impairments in tissue regeneration with age (Signer et al., 2013). Interestingly, reducing $p16^{INK4A}$ expression in these old keratinocytes restored the normal thickness of the epidermis, similar to that formed by young keratinocytes.

Besides deleterious effects on physiological parameters, aging affects psychological state. In fact, it has been shown that aging has negative effect on mood and Self Esteem, self-esteem being all about how much you feel you are worth — and how much you feel other people value you. It is also seen that many woman attaches self esteem to their body image which is associated with beauty, femininity and youth so with growing age as beauty diminishes many a woman find their self worth low and thus giving rise to a low self esteem Pearlman (1993).

Slow down aging by acting on the senescence process may be beneficial for the overall health, and the development of specific interventions that target senescent cells may serve as a therapy to delay aging, including skin pathologies and mood (Velarde and Demaria, 2016). The cosmetics industry has focused on bioactive substances derived from natural products such as plants, mushrooms, and marine algae. Besides these products, bacteria such as *Lactoccus lactis* strain may prevent immune-senescence and decelerates individual senescence (Tsuji et al., 2018). Another genus, such as the genus *Sphingomonas*, hallmarked by their oligotrophic nature and plasticity in man-made environments, has been intensively exploited for their metabolic properties relevant to biotechnological importance (Gulati et al., 2017). It exhibits a yellow-pigment coloration due to the presence of two enzymes, a catalase and an oxidase, which allow it to produce a carotenoid pigment named nostoxanthin. The precise function of this unique carotenoid in this type of micro-organisms is likely associated with tolerance to environmental stress due to the antioxidant activity of carotenoids. The second specificity of this bacteria is to contain glycosphingolipids (GSLs) instead of lipopolysaccharide (LPS) in their cell envelopes. The GSLs appear to act as a barrier to bactericidal substances (Kawahara et al., 1999). During the past 10 years, *Sphingomonas* strains have been isolated from a variety of environments including both aqueous (both fresh- and seawater), terrestrial habitats and plant root systems (Aylward et al., 2013). The widespread distribution in the environment is due to its ability to utilize

a wide range of organic compounds and to grow and survive under low-nutrient conditions. These bacterial species described in the environment may play a role in skin homeostasis. This is the One-Health concept, which recognizes that the health of humans is connected to the environment (Lee et al., 2001).

Based on its rich and unique composition, *Sphingomonas* sp. represents an innovative source for the development of new skin care solutions. In this study, we firstly aimed to investigate the effect of *Sphingomonas* sp. on expression of $p16^{INK4a}$ and $p21^{WAF1}$ and SA-β-gal activity using an aged 3D human skin model, a full-thickness skin model engineered with aged fibroblast treated during the tissue reconstruction. Because the SASP is characterized by the secretion of inflammatory signals that resembles a local immune response, the capability of the bacteria extract to modulate the expression of inflammatory cytokine (IL-6) or chemokine (IL-8) were investigated on Normal Human Dermal Fibroblats. Finally, an *in vivo* study was carried out among 24 women aged from 60 to 70 years to evaluate the effect of this bacteria extract on skin and on psychological state. Based on the beneficial properties of microorganisms and the plasticity of bacterial genomes allowing bacteria to adapt quickly to environmental conditions, we put forward the hypothesis that *Spinghomanas* sp. could slow down the cell senescence mechanism.

Materials and Methods

To conduct such research, skin equivalent model was engineered with aged Normal Human Dermal Fibroblasts (NHDF) and treated with the bacterial extract at different concentrations, in a systemic way for 42 days. As a preliminary evaluation, cytotoxic analysis on cell culture monolayers were conducted to select the highest non-cytotoxic concentrations and to avoid any cumulative deleterious effect on 3D reconstructed skin model. Several histological and immunohistological analysis were made: study of the elasticity of the dermal compartment by the analysis of the expression of the fibrillin and versican proteins. Finally, the fibroblast senescence was investigated by the analysis of the p16, p21 expression, SA-β-gal, IL-6 and IL-8.

A clinical evaluation of this active ingredient was also performed in France (Lyon) in open, intra-individual study by comparison before and after hemi-face application of this extract twice a day. 24 subjects were involved from 60 to 70 years old. The study was performed during 56 days. Cutaneous hydration rate, Trans Epidermal Water Loss, skin biomechanical properties, cutaneous relief parameters, and orientation of the lines in the cutaneous relief were evaluated.

Besides, self-esteem was evaluated at D0 and D56 using the Rosenberg scale (1965). The scale is a ten item Likert scale with items answered on a four point scale – from strongly agree to strongly disagree. The Profile of Mood States (POMS) which is a psychological rating scale used to assess transient, distinct mood states, was also administered at the same period (Mc Nair et al.1971).

Results

Sphingomonas extract treatment at a concentration of 0.1% allowed decreasing p21 expression in the dermis of reconstructed skin. This decrease was about 58% ($p<0.001$) versus untreated condition (Figure 2). We also noted a significant decrease in the p16 expression. This decrease is respectively 20% versus the untreated condition (★★★ $p<0.001$).

Figure 3 shows the expression of β-galactosidase. Retinoic acid and *Sphingomonas* extract treatment decreased the β-galactosidase expression as compared to the untreated control. These decreases were 78% versus the untreated condition ($p< 0.01$).

We know that exposure to lipopolysaccharide (LPS) results in the formation of hydrogen peroxide in a concentration-dependent manner and that LPS induce cellular senescence and secretion of IL-8 and IL-6 as noted in the literature. The bacteria extract induced a significant decrease of IL-8 when compared with LPS ($p<0.01$) and a decrease of 31% when compared with the untreated condition. This observation was also reported for IL-6.

Efficacy has also been demonstrated on the synthesis of extracellular matrix components (neocollagen, fibrillin-1, versican).

Finally, after 56 days of *Sphingomonas* extract treatment, we noted a significant improvement in average roughness (-9% $p=0.025$ versus D0, with a positive effect for 68% of subjects) and average relief (-8% $p=0.044$ versus D0, with a positive effect for 64% of subjects). The skin was also noticeably more supple. In fact, we noted a significant increase in Ue parameter (reflecting the suppleness evaluated using the deformation and immediate extensibility of the skin) of +12% on average ($p< 0.001$ versus D0), effect observed in 70% of the subjects. At the same time, the placebo induced a non significant increase of 4%.

Using a VISIA from CANFIELD® imaging system, which allows to take pictures with different types of illuminations and a very rapid capture of images, we reported that this active ingredient induced anti-wrinkle effect on crow's feet (Figure 4).

Concerning the psychological status, we noted a significant increase of self-esteem between D0 and D56 (10.5%, $p< 0.001$; 31.3 ± 0.7 versus 34.6 ± 0.67 respectively). At the same time, the mood state also significantly increased ($p< 0.003$).

Discussion and Conclusion

Understanding the mechanisms that underlie senescence and skin is of importance not only for cosmetic purposes, but also from a biomedical viewpoint considering to avoid autoimmune diseases, and various malignancies. Considering the aging population, there is a fear of a significant increase in cases in the coming years. Research of natural substances that can delay skin aging has been the object of increasing interest in the last few

years. Recently, probiotic bacterial fermentation emerges as one of crucial processing tools in cosmetic technologies in order to enhance absorption into the skin, improve desirable pharmacological activities. In contrast to probiotic extract, little has been done on other microorganisms.

To our knowledge, we are the first to identify that *Sphingomonas* extract delays intrinsic skin aging process by inhibiting cellular senescence. In fact, it does not only attenuate cellular senescence through inhibition of the p21 and p16 and signaling pathways but also increase the expression of fibrillin-1, implicating in the formation of elastic fibers and elastin. The *in vivo* study showed that this bacteria extract maintains skin's beauty after 60 years old by maintaining the cutaneous barrier and by improving the suppleness of the skin. It has also the capability to restructure and has and anti-aging effect.

As suggested by Pearlman (1993), physical changes between the ages of 55 and 60 years affect one's physical and disrupt self-esteem. The present findings showed that the benefical physiological effects induced by SP has a positive influence on mood and self-estem. Because skin aging causes emotional distress, *Sphingomonas* extract can serve as an anti-aging cosmeceutical agent and help to build a better psychological health.

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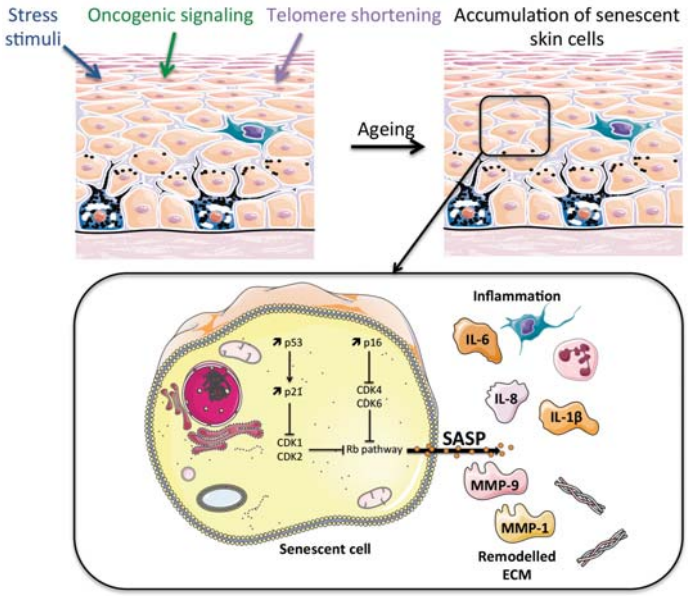

Figure 1 : Causes and consequences of senescence
The senescence phenotype is induced by multiple stimuli which activate p53/p21 or p16 pathways according to the factors that cause stress. p16 is found to be major CDK inhibitor for both CD4 and CDK6 kinases. p21 binds to and inhibits the activity og cyclin-CDK2, CDK1 inducing growth arrest. Senescent cells release proinflammatory agents including IL-6, TNF-α and IL-8, matrix metalloproteinase-9, cyclooxygenase-2…which are prominent sources of ROS and accelerate the ageing process.
CDK: Cyclin-dependent kinase

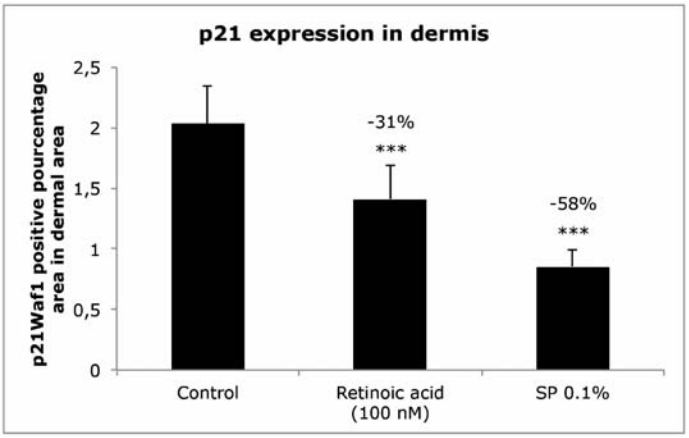

Figure 2 – p21Waf1 expression quantification normalized by the total dermal area. Automatic analysis of staining with « ImageJ » software. Statistical analysis: t-test. *** p<0.001.

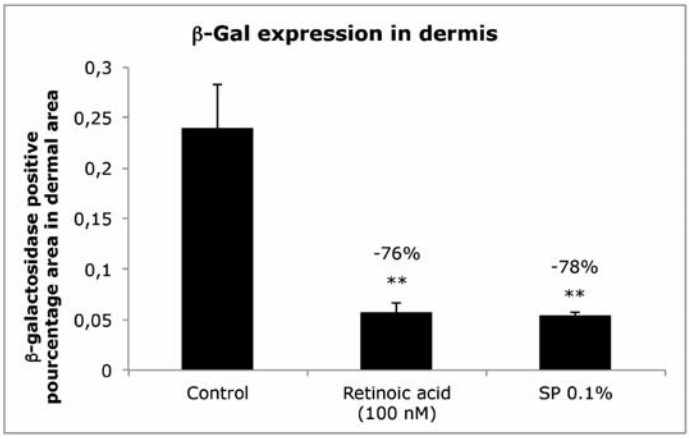

Figure 3 – Quantification of b-galactosidase positive cells in the dermis of aging reconstructed skins. Automatic analysis of staining with « ImageJ » software. Statistical analysis: t-test. ** p<0.01.

Figure 4 – Pictures of volunteer 4 at D0 and D56 with the use of Sphingomonas extract

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